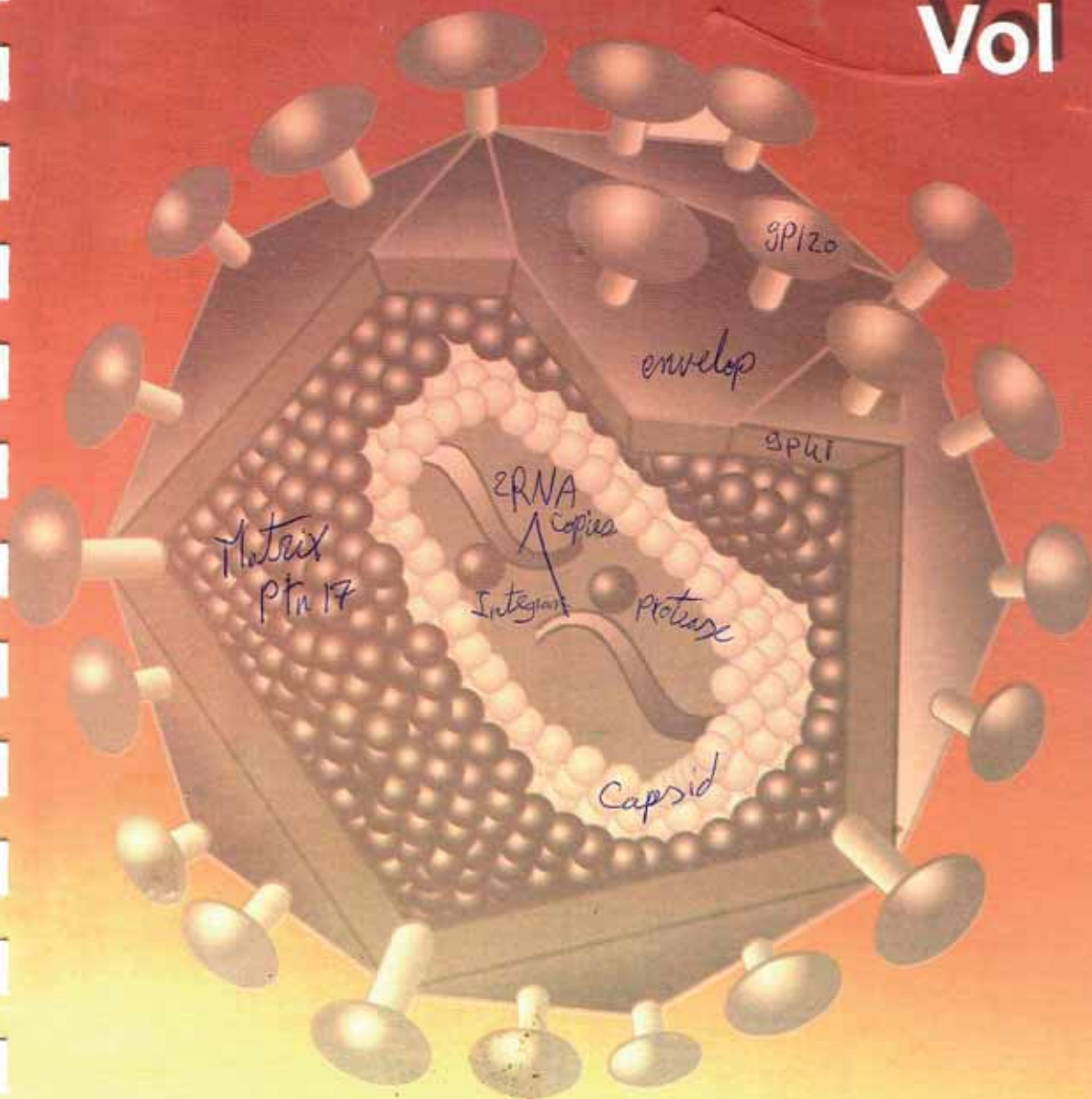


Essential Medical Microbiology and Immunology

Vol III



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Chapter 1

GENERAL VIROLOGY

Viruses are one of the smallest infectious agents. They are obligatory intracellular parasites because they have no metabolic activity.

Viruses can infect all organisms in nature:

1. Bacteriophages: are bacterial viruses.
2. Plant viruses: include complete viruses and viroids.
3. Animal viruses: infect insects or vertebrates including man.

RNA ptm free fragment circular

Viruses differ from bacteria in the following:

1. Viruses are very small in size, ranging from 10-300 nm. So:
 - They can only be seen under the electron microscope (except poxviruses).
 - They can pass through bacterial filters.
 - They need ultracentrifugation for sedimentation.
2. Viruses contain only one type of nucleic acid (DNA or RNA), never both.
3. They are obligatory intracellular parasites, i.e. can only replicate inside living cells. They do not divide by binary fission.
4. They can not be cultivated in the laboratory on artificial culture media.
5. They are not susceptible to antibacterial antibiotics.

Structure and Composition of Viruses

The typical complete virus particle, called **virion**, consists of a **genome** of either DNA or RNA, surrounded by a **capsid** (protein coat), which is composed of many small protein subunits called **capsomeres**. The nucleic acid and the protein coat are called **nucleocapsid**. Some viruses, called enveloped viruses, have an outer envelope composed of lipids and polysaccharides, other viruses are non-enveloped (Fig.1&2).

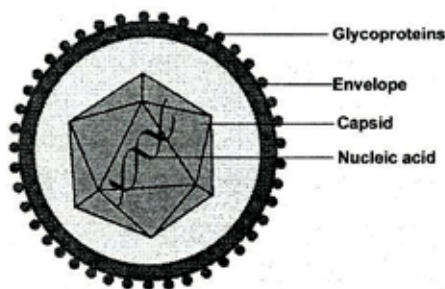


Fig. (1): Diagram illustrating the components of the complete virus particle (virion).

Viral capsid

Viral capsid has the following functions:

1. It protects the nucleic acid (genome).
2. It mediates attachment to host cell (in non-enveloped viruses).
3. It is the antigenic part of the virus.
4. It is responsible for the viral morphology (or symmetry).

Viral symmetry

Electron microscopic (E.M.) examination shows that viruses have 3 types of symmetry (Fig. 2):

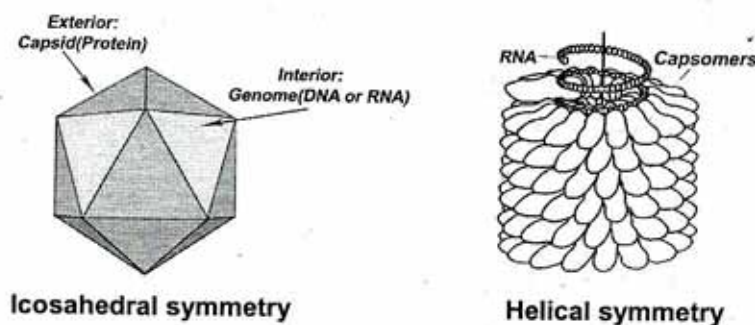


Fig. (2): Virus Symmetry

- 1. Icosahedral (many sided) symmetry:** Icosahedral or isomeric or cubic viruses resemble a crystal with 20 triangular facets and 12 corners. This include all DNA viruses, except poxviruses (brick shaped), and some RNA viruses.
- 2. Helical (coiled tubes) symmetry:** The viral nucleic acid is closely associated with the protein capsid forming a coil-shaped helical nucleocapsid. This includes many of RNA viruses, e.g. rabies virus.
- 3. Complex symmetry:** e.g. the brick-shaped poxviruses.

Viral nucleic acid (genome)

It is responsible for virulence, i.e. it is the infectious part of the virus. Also, it carries the genetic information of the virus.

Only one type of nucleic acid is present in the virus, DNA or RNA. Most DNA viruses are double-stranded (ds) while most RNA viruses are single-stranded (ss). The viral ssRNA may be positive sense strand (+sense) or negative sense strand (-sense).

Viral envelope

It is formed of lipids or lipoproteins and is derived partly from the host cell membrane during release by budding.

It is sensitive to lipid solvents, e.g. ether. It has glycoprotein spikes which are the organ of attachment of the enveloped virus to host cell receptors.

Virus Replication

Viruses are unable to replicate on their own because they lack the genes and enzymes necessary for energy production. Therefore, replication depends on living host cells and is directed by the viral genome to produce the virus components. Viral replication occurs in the following steps:

- 1. Attachment or adsorption:** Adsorption of the virus occurs to **specific receptor** sites on the surface of the susceptible host cell (**tropism**). This step is highly specific.

and explains why certain viruses cause respiratory infections while others cause gastrointestinal infections.

2. Penetration: The entire virus enters the cell by:

- a- Endocytosis as in case of non-enveloped viruses.
- b- Fusion of viral envelope with host cell membrane only in case of enveloped viruses.

3. Uncoating: The nucleic acid is released from the capsid by lysosome enzymes and is available for replication.

4. Eclipse: This is the time from uncoating until assembly of mature viruses. During this phase, no infectious viruses can be detected in the host cell.

6. Synthesis of viral components: Viruses must first synthesize messenger RNA (mRNA). Specific messenger RNAs are transcribed from the viral nucleic acid, and are translated in the cell ribosomes to form viral components. Transcription of mRNA varies according to the type of viral nucleic acid whether DNA or RNA, ds or ss, positive or negative sense strand, as follows:

- a) **DNA viruses:** in which mRNA can be formed using the host's own RNA polymerase to transcribe from negative sense strand.
- b) **RNA viruses:** These are 4 groups of RNA viruses in which RNA cannot be transcribed like DNA viruses, as host polymerases do not work from viral RNA. The virus must provide its own polymerases. RNA viruses produce mRNA as follows:
 - In dsRNA viruses, one strand is first transcribed by viral RNA-dependent RNA polymerase (RdRp) into mRNA.
 - In ssRNA viruses there are 3 distinct routes to the formation of mRNA:
 - i. The strand with positive sense acts directly as mRNA.
 - ii. The strand with negative sense must first be transcribed, using viral RdRp into positive sense strand, which can then act as mRNA.
 - iii. Retroviruses which contain positive ssRNA, by the action of reverse transcriptase will produce complementary ssDNA. Thus, it is converted to dsDNA which enters the nucleus and is either integrated in host cell genome causing transformation or is transcribed by host polymerase into mRNA.

On the other hand, viruses must also replicate their nucleic acid.

Also it should be noticed that some viruses do not initiate synthesis and remain latent within the host cell for variable periods.

6. Assembly: The nucleic acids are enclosed within the protein coats to form mature viruses (virions). This occurs either in the nucleus of host cell, e.g. herpes viruses or in the cytoplasm, e.g. polioviruses.

7. Release: New viruses are released either by:

- a. Lysis of host cell in case of non-enveloped viruses, e.g. poliovirus.
- b. Budding through the cell membrane in case of enveloped viruses, e.g. HIV.

Cultivation of Viruses

There are three main methods for cultivation of viruses in the laboratory:

- I. Cell culture (tissue culture).
- II. Embryonated eggs.
- III. Laboratory animals (intact animals).

I. Cell culture (tissue culture)

Pieces of tissues are treated with trypsin to get separate cells. These cells are then grown in bottles, tubes or plates to form attached monolayer culture. Viruses are inoculated on this sheet of cells and incubated at 37°C for 24-48 hours.

Types of cell culture: There are three types of cell lines:

1. **Primary cell lines:** Prepared from organ fragments, e.g. monkey kidney. They have a limited number of subcultures (5-10 passages).
2. **Diploid cell lines:** Prepared from human embryo tissue, e.g. lung. They can be subcultured for 50-100 passages.
3. **Continuous cell lines:** Prepared from tumour cells with unlimited number of subcultures or passages, e.g. HeLa cells from carcinoma of cervix.

Detection of virus replication in cell culture: Virus growth could be detected by:

1. **Cytopathic effect (CPE):** CPE are the morphological changes in the infected cells seen microscopically. This occurs with most viruses and includes:
 - a) Cell death or lysis.
 - b) Syncytial formation (giant cells): due to fusion of membranes of adjacent cells to form multinucleated giant cells, e.g. respiratory syncytial virus (RSV), measles and mumps viruses.
2. **Inclusion bodies:** These are either the site of virus assembly or degenerative changes in the cell. They are used as a diagnostic tool to identify certain viral diseases. They may be:
 - a- Intracytoplasmic: e.g. poxviruses and rabies (Negri bodies).
 - b- Intranuclear: e.g. herpes viruses.
 - c- Both: e.g. measles virus.
3. **Plaque formation:** Plaques are areas of virally-infected cells in a monolayer cell culture. They are seen by the naked eye as unstained areas when using a vital dye.
4. **Transformation:** The nucleic acid of the virus gets incorporated with the genetic material of the cell making it transformed e.g. herpes virus and other oncogenic viruses. This could be detected by foci of malignant cells in the cell line used.
5. **No change for several weeks, e.g. rubella virus:** This could be detected by its ability to interfere with the growth of another CPE- producing virus added as an indicator (interference phenomenon).
6. **Haemadsorption:** Haemagglutinating viruses, e.g. influenza viruses are detected by adding suspension of RBCs to the infected cell line. The RBCs will be clumped only on the virally-infected cells.

7. Direct fluorescent antibody staining of infected cells.

8. Detection of viral antigens released in the growth medium can be detected by any serological test, e.g. complement fixation (CF), haemagglutination (HA)... etc.

9. Neutralization test: This can be done by the use of specific antiviral antisera which will block or neutralize the infectivity of the virus.

II. Embryonated eggs

It contains a variety of membranes that support multiplication of certain viruses.

III. Laboratory animals

It was the first method used for cultivation of viruses. It is still used in the following:

1. Isolation of coxsackie viruses and togaviruses by inoculation in suckling mice.
2. Rabies virus inoculation in mice.
3. For research.

Classification of Viruses

A- Classification by symptomatology

It is the old classification based on diseases they produce, i.e. tropism, e.g. neurotropic viruses, enteroviruses, ... etc.

B- The Hierarchical virus classification: It is a scheme classifying viruses into order, family, subfamily, genus, species, type, subtype, and strain. The criteria used to differentiate virus orders, families, and genera are:

1. Nature of the nucleic acid: RNA or DNA genome
2. Virus replication strategy
3. Symmetry of the capsid
4. Presence or absence of an envelope
5. Size of the virion and capsid

C- The Baltimore Classification: It is based on virus genome replication strategy. The central idea is that all viruses must generate positive strand mRNAs from their genomes, in order to produce proteins and replicate themselves. The precise mechanisms whereby this is achieved differ for each virus family.

Pathogenesis of Viral Diseases

I. Entry of viruses

Viruses enter the body either by inhalation (respiratory tract), ingestion (gastrointestinal tract), contact (urogenital system) or through skin (injections, blood transfusion, insect and animal bites).

Viruses usually replicate in the primary site of entry. Some viruses produce disease at the portal of entry (local infections), others have to spread to distant organs either via the blood (viraemia), or by other means, e.g. along nerves and produce systemic or deep viral infections (Table 1).

Table (1): Differences between local and systemic viral infections.

	Local infections	Systemic infections
Specific disease	Respiratory	Measles
Example	(common cold e.g. rhinovirus)	
Site of pathology	Portal of entry.	At distant sites
Incubation period	Relatively short	Relatively long
Viraemia	Absent	Present
Duration of immunity	Usually short	Usually lifelong
Role of secretory IgA antibody in immunity	Usually important	Usually not important

II. Fate of viral infections

1. Inapparent or subclinical viral infections: Viral infection without overt signs and symptoms.

2. Apparent infections (disease): This may be local or systemic with the appearance of clinical signs and symptoms.

3. Persistent viral infections (chronic): In this form, the virus is continuously detected with mild or no clinical symptoms, e.g. chronic hepatitis B.

4. Latent viral infections: The virus persists in a dormant form and may flare up intermittently to produce disease, e.g. herpes viruses.

5. Slow virus infections: Virus infections with long incubation periods (months or years). They are caused by two types of infectious agents:

1. Conventional viruses, e.g. a variant of measles virus which causes subacute sclerosing panencephalitis (SSPE).
2. Unconventional agents (prions).

Laboratory Diagnosis of Viral Infections

I. Direct detection of viruses and/or their components: The following procedures are used:

1. Light microscopy: The ordinary microscope can be used in examination of large viruses as poxviruses, or giant cells in herpes infection, or inclusion bodies, e.g. Negri bodies in nerve cells in rabies.

2. Fluorescent microscopy: By using direct immunofluorescent antibody technique (IF), e.g. diagnosis of rabies in brain smears.

3. Electron microscopy (EM): This method is used when large number of viruses is present in the sample. It also gives an idea about the size and shape of viruses.

4. Immunoelectron microscopy (IEM): This is done by the addition of specific antibodies to the clinical sample. This will lead to aggregation of the unknown virus particles. It is used to detect viruses that cannot be cultured, e.g. rota virus and hepatitis A virus in stools.

5. Immunoassays: For detection of the virus antigens by the use of either ELISA or RIA; e.g. hepatitis B antigens in blood.

6. Nucleic acid hybridization: It is a highly sensitive and specific method. Specific probes prepared and labeled are added to clinical samples. It will hybridize with the complementary nucleic acid of the virus in the specimen.

7. Polymerase chain reaction (PCR): It is a recent method in which amplification of a short sequence of a target nucleic acid of the virus allows it to be easily detected by different methods, e.g. probes.

II. Isolation of viruses

- The specimen is taken from sites or lesions suspected to contain the virus.
- Antibiotics are added to prevent bacterial contamination. Then centrifugation is done and the supernatant is used for isolation of viruses.
- Isolation of virus is done by inoculation of cell culture, embryonated egg or laboratory animals.
- The choice will vary according to the suspected virus.

III. Serological diagnosis

- It is an indirect method to detect antiviral antibodies.
- Usually 2 serum samples (paired serum) are taken. The first in the acute phase and the second 2-3 weeks later, to demonstrate a rising titre (4 fold increase or more is diagnostic).
- Only one sample may be used in the acute stage to detect IgM, e.g. in diagnosis of rubella in early pregnancy.

The serological methods used are: Neutralization test (NT), complement fixation test (CF), haemagglutination inhibition test (HI), enzyme-linked immunosorbant assay (ELISA), radioimmunoassay (RIA) and indirect immunofluorescence (IIF).

IV. Skin tests can be used as an indication of cell-mediated immunity (CMI) in some viral infections, e.g. mumps.

Treatment of Viral Infections

Viruses can not be treated with antibiotics because they lack the structural targets on which antibiotics can act. Viruses are obligate intracellular parasites, so antiviral drugs must selectively inhibit viral replication without causing damage to host cells. The number of antiviral drugs is little compared to antibacterial drugs (Table 2):

Table (2): Selected antiviral drugs and their mechanism of action and clinical use

Mechanism of action	Antiviral drugs	Virus infections Examples of viral diseases
I. Fusion inhibitors that block virus entry	Fuzeon (enfuvirtide)	HIV
II. Inhibition of uncoating of viruses	1. Amantadine	Influenza A virus
	2. Rimantadine	Influenza A virus (less toxic)
III. Neuraminidase inhibitors interfere with production and release of virus from cells of respiratory tract.	1. Oseltamivir (Tamiflu)	▪ Influenzavirus A ▪ Influenzavirus B
	2. Zanamivir	▪ Influenzavirus A ▪ Influenzavirus B
IV. Nucleoside analogues that inhibit DNA polymerase; and consequently inhibit DNA synthesis	1. Acyclovir	▪ Topical: herpes simplex virus (HSV) type 1 and 2 and varicella-zoster virus ▪ Parenteral: treatment of hepatitis B virus (HBV) infection
	2. Ganciclovir	Cytomegalovirus
	3. Vidarabine	Herpes viruses, HBV
	4. Iododeoxyuridine	herpetic keratoconjunctivitis
V. Inhibition of mRNA synthesis	Ribavirin	Respiratory syncytial virus and influenza B infection
VI. Nucleoside analogues that inhibit DNA synthesis by inhibiting viral reverse transcriptase enzyme	1. Azidothymidine	HIV
	2. Zalcitabine	HIV
	3. Lamivudine	HIV & HBV
	4. Stavudine	HIV
VII. Protease inhibitors that inhibit cleavage of precursors polypeptide	1. Indinavir	HIV
	2. Ritonavir	HIV
	3. Saquinavir	HIV
VIII. Inhibition of viral protein synthesis	1. Methisazone	Poxviruses (smallpox)
	2. Interferons	▪ Chronic hepatitis B & C ▪ Human papilloma virus

Chapter 2

HEPATITIS VIRUSES

There are many different viruses that cause infection of the liver, which may be the primary target or secondary target organ as shown in table (3).

Table (3): Viruses infecting the liver and their taxonomy

	Virus name	Gemone	Envelope	Taxonomic list	
				Genus	Family
Primary Hepatotropic	Hepatitis A virus (HAV)	(+)ssRNA	No	Hepatovirus	Picornaviridae
	Hepatitis E virus (HEV)	(+)ssRNA	No	Hepevirus	Hepeviridae
	Hepatitis B virus (HBV)	dsDNA-RT (partial)	Yes	Hepadnavirus	Hepadnaviridae
	Hepatitis delta virus (HDV)	(-)ssRNA	Yes	Deltavirus	NA
	Hepatitis C virus (HCV)	(+)ssRNA	Yes	Hepacivirus	Flaviviridae
?	Hepatitis G virus/ GB virus type C (GBV-C)?	(+)ssRNA	Yes	NA	Flaviviridae
Secondary Hepatotropic	Human cytomegalovirus (CMV)	dsDNA	Yes	Cytomegalovirus	Herpesviridae
	Epstein-Barr virus (EBV)	dsDNA	Yes	NA	Herpesviridae
	Yellow fever virus (YFV)	(+)ssRNA	Yes	Flavivirus	Flaviviridae

*envelope formed of hepatitis B surface antigen; NA, not assigned; RT, reverse transcriptase

?there is a debate whether GBV-C virus is primarily hepatotropic or lymphotropic.

Hepatitis A Virus (HAV)

HAV causes hepatitis A which has been called infectious hepatitis in the past. The disease occurs in sporadic or epidemic forms. There is only one serotype and development of antibodies to HAV confers lifelong immunity. Taxonomy is mentioned in table (3).

Pathogenesis

The host range for this virus is limited to humans and a few other primates. The virus is transmitted mainly by the faeco-oral route usually through ingestion of contaminated food and water. HAV replicates in cytoplasm of hepatocytes, and then is shed in bile, which results in high titer of infectious HAV in faeces. It affects children and young adults. Increased risk of transmission is due to decreased sanitary hygienic factors.

Clinical features

The incubation period is 2-6 weeks. Asymptomatic and subclinical infections are common in children. Viral hepatitis may be preceded by generalized gastrointestinal symptoms and fever. Nausea, vomiting, and anorexia are common symptoms. Many cases are without jaundice. Hepatitis A infection is usually mild, self-limited and does not progress to chronic hepatitis.



Laboratory diagnosis

1. Marked elevation of liver transaminases and bilirubin.
2. Detection of anti-HAV IgM (by RIA or ELISA) is diagnostic of acute phase.
3. Detection of anti-HAV IgG during convalescence. It indicates immunity and may persist for decades.
4. Detection of HAV particles in stools or blood by RIA, PCR, genetic probes, or electron microscope.

Prevention and control

1. Proper hand-hygiene, chlorination, or boiling of drinking water.
2. Careful disposal of sewage and avoiding contamination of food and water.
- ③ **An inactivated vaccine (Havrix)** can be given intramuscularly (IM) as two doses at 0 and 6 months. It is recommended to high-risk individuals including children above 2 years of age who live in endemic countries.
- ④ HAV immune globulin for post-exposure prophylaxis to prevent disease in the immunodeficient.

Hepatitis E Virus (HEV)

HEV is characterized by faeco-oral transmission and a short incubation period of about 6 weeks. Disease is of self limited nature occurring either sporadic or in the form of epidemics. It affects mainly young adults. There is little risk of development of chronic hepatitis, but there is a high mortality rate due to disseminated intravascular coagulation especially in pregnant females.

Diagnosis

- a) PCR is used to detect viral RNA in stools.
- b) Detection of anti-hepatitis E virus IgM and IgG by ELISA.

Prevention and control: a large trial in China has found an experimental vaccine appears to be safe and effective in protecting people against hepatitis E infection

Hepatitis B Virus (HBV)

HBV is a DNA virus of *Hepadnaviridae* family (Table 4) that causes serum hepatitis. The intact virion, known as the Dane particle (Fig. 5), is spherical with 42 nm diameter, and is composed of:

1. **An outer shell of lipoprotein containing the hepatitis B surface antigen (HBsAg):** HBV expresses excess HBs antigen (protein) and releases it into the bloodstream as independent particles (not always associated with DNA). There are two forms of such particles, 22 nm spherical and 200 nm tubular particles which are non-infectious. Therefore, large amounts of HBsAg circulate in the blood during infection and carrier state. HBsAg stimulates production of neutralizing antibodies, thus, providing long-term protection against HBV infection.

2. A core contains the hepatitis B core antigen (HBcAg), hepatitis B e antigen (HBeAg) and DNA-dependent DNA polymerase.
3. The virus genome is a partially double-stranded DNA.

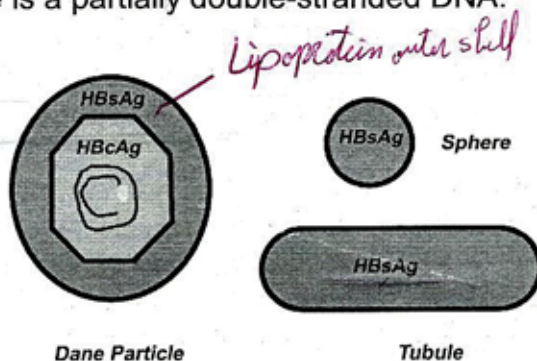


Fig. (5): Structure of HBV. *42 nm diameter*

Modes of HBV transmission

HBV is present in body fluids of infected individuals. *G.R. HBV transmit faster* Because viral load is very high and may reach 10^{10} copies per mL of serum, the rate of transmission may be very high compared to the bloodborne viruses HIV and HCV. The main routes of infection are perinatal transmission, blood and percutaneous transmission, and sexual transmission (see table 5 for the modes of transmission and high-risk groups).

Pathogenesis and clinical presentations

HBV is a hepatotropic virus that replicates in the liver and causes hepatic dysfunction. Antibody to HBsAg and HBeAg can block binding of virions to hepatocytes, but large amounts of antigen in the bloodstream bind up the antibody and inactivate it. *G.R. extrahepatic lesion of HBV?* Extra-hepatic manifestations similar to serum sickness (rash, urticaria, and polyarthralgia), vasculitis and glomerulonephritis can result from immune complex deposition. *Due to Immune Complex deposition type 3 Hypersensitivity*

- The incubation period ranges from 6 weeks to 6 months.
- The onset of the disease is gradual.
- The clinical manifestations depend on the age at infection, level of HBV replication, and host's immune status:
 1. Perinatally infected infants generally have no clinical signs or symptoms, and infection produces typical illness in only 5-15% of children aged 1-5 years.
 2. Older children and adults are symptomatic in 33-50% of infections.
- Constitutional symptoms such as malaise and anorexia may precede jaundice by 1-2 weeks.
- Clinical symptoms and signs include nausea, vomiting, abdominal pain, and jaundice. Skin rashes, joint pains, and arthritis may occur. The liver is enlarged and tender.
- Most patients recover fully and spontaneously within 4-6 months.
- Fulminant hepatitis occurs in 1-2% of patients with acute disease. Hepatic failure occurs in approximately 0.1-0.5% of patients and is believed to be caused by massive immune-mediated lysis of infected hepatocytes.
- Chronic infection can lead to long term complications:
 - Chronic active hepatitis and cirrhosis that end in liver failure and death.
 - Hepatocellular carcinoma.

Laboratory diagnosis (Fig. 4 and table 4)

A) **Hepatitis B virus markers:** These are HBV antigens and antibodies:

1. **HBsAg:** It can be detected in the blood during the incubation period and active disease. It usually declines within a period of 12 weeks.

Its persistence for more than 6 months indicates a chronic carrier state.

2. **Anti-HBs:** It appears late, with the disappearance of HBsAg, and denotes immunity.

3. **HBcAg:** It is present only in the liver cells, and can not be detected in blood.

4. **Anti-HBc:** It is the antibody to the HBcAg. Its two immunoglobulin classes IgM and IgG plays a key role in diagnosis of the different events of HBV infection. IgM anti-HBc is detectable at the time of clinical onset and declines within 6 months, thus it is a valuable diagnostic test for recent HBV infection when other markers are negative.

There is a period designated the **window phase** during which HBsAg has disappeared while anti-HBs is not yet detectable. During this phase, IgM anti-HBc is always positive and diagnostic.

IgG anti-HBc persists indefinitely as a marker of past infection.

5. **HBeAg:** It can be detected in the serum in the late incubation period and during the acute illness. Its presence is associated with high infectivity of the patient. Its disappearance is a good prognostic sign.

6. **Anti-HBe:** Its detection is a strong evidence of recovery. Persistent HBeAg and absence of HBeAb is an indication for treatment, as such a patient is developing chronic active hepatitis.

B) **DNA polymerase** activity: This can be demonstrated during viraemic stage of HBV infection.

C) **Viral DNA:** can be detected by probe or PCR. It is indicative of viral replication and is important in diagnosis of chronic infection.

D) **Liver function tests**, e.g. serum alanine aminotransferase (ALT) and bilirubin, supplement the diagnosis. Liver biopsy permits a tissue diagnosis of hepatitis.

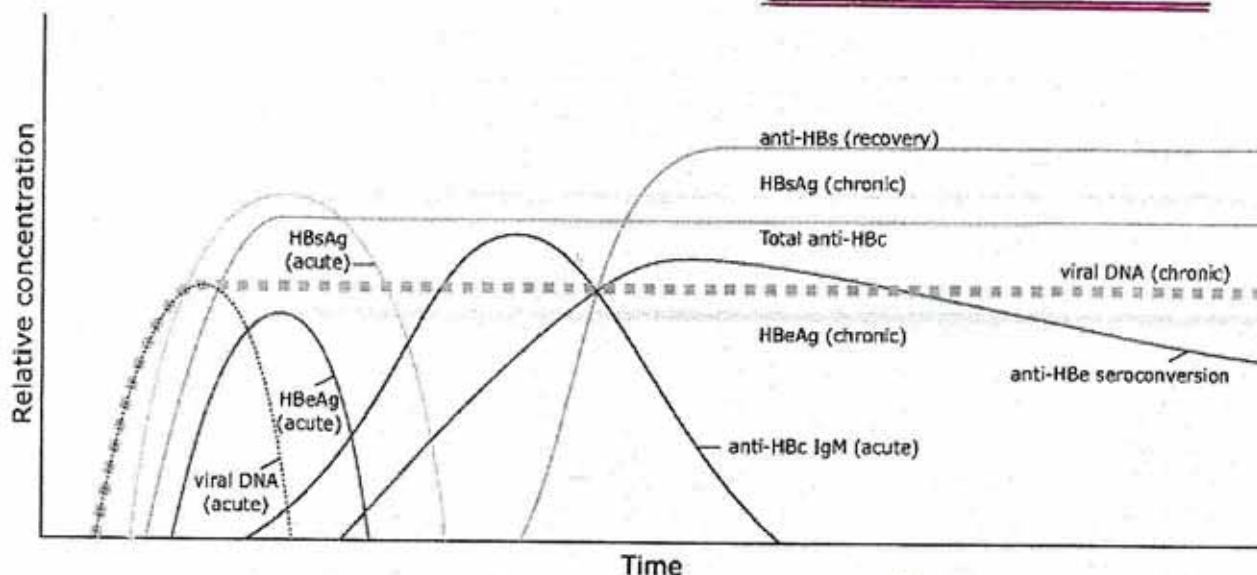


Fig. (4): Typical serologic course of HBV infection

HBsAg → Incubation
chronicity
↓ HBsAg = recovery

Table (4): Interpretation of hepatitis B serology

Tests	Results	Typical interpretation	not Diagnostic
HBsAg anti-HBc anti-HBs	Negative Negative Negative	<u>Susceptible individual</u>	bec. it's mostly empty shell
HBsAg HBeAg DNA polymerase HBV DNA	Positive Positive Positive Positive	<u>Viraemia stage</u>	HBV DNA + polymerase
HBsAg IgM anti-HBc anti-HBs	Positive Positive Negative	<u>Acute</u> HBV infection	IgM Anti HBc + HBsAg (no HBs)
HBsAg IgM anti-HBc anti-HBs	Negative Positive Negative	<u>Window phase</u>	Define = period HBsAg disappear & no Anti HBsAg detect while IgM anti HBc (+ve) = diagnostic
HBsAg IgG anti-HBc IgM anti-HBc anti-HBs	Positive Positive Negative Negative	<u>Chronic</u> HBV infection	S + IgG (no IgM)
HBsAg IgG anti-HBc anti-HBs	Negative Positive Positive	<u>Immune</u> individual following <u>natural</u> infection	
HBsAg anti-HBc anti-HBs	Negative Negative Positive	<u>Immune</u> individual following <u>HBV</u> vaccination	

Treatment

No specific treatment is available for acute illness. Antiviral drugs are approved for the treatment of chronic hepatitis B.

Prevention and control

- General measures** directed at preventing exposure are important as:
 - Screening of blood before transfusion.
 - Implementing** the Infection Control Practices of the **Standard Precautions** such as hand hygiene and use of gloves (see chapter on infection control).
 - Use of disposable syringes, tattoo needles ... etc.
 - Avoid recapping of the used needles and injury with sharp instruments.

2. Specific prophylaxis:

a- Hepatitis B vaccines: These are recombinant vaccines produced by using HBsAg synthesized by yeast, into which a plasmid containing the gene for HBsAg has been inserted. Purified HBsAg is obtained by lysing the yeast cells. HBsAg protein is then adsorbed to alum.

The vaccine is given in series of three intramuscular doses at 0, 1, 6 months. The series of three doses induces a protective antibody response. The vaccine should be administered only in the deltoid muscle of adults and children or in the anterolateral thigh muscle of neonates and infants.



b- **Hepatitis B immunoglobulin (HBIG)** together with vaccination are given as **post-exposure prophylaxis** to:

1. Neonates born to HBsAg positive mothers.
2. Those exposed to HBV but unvaccinated or without vaccine response.

Hepatitis Delta Virus (HDV)

The hepatitis delta virus is a unique defective spherical 36 nm virus, composed of a core containing a circular, ss-RNA genome, surrounded by HB surface antigen. HDV can only replicate in patients with HBV infection.

Infection is either a coinfection where the patient acquires both viruses at the same time or a superinfection, i.e. on top of a chronic HBV infection where it may lead to an acute exacerbation.

As HDV is dependent on HBV it follows a similar epidemiology. It can be controlled through control of hepatitis B infection.

Diagnosis

By detection of anti HDV antibodies

Hepatitis C Virus (HCV)

6 genotypes
type 4A in Egypt
very rare

HCV infection is a growing major health problem worldwide and in Egypt, 50% of HCV infections are associated with development of chronic **active** hepatitis that ends in cirrhosis or hepatocellular carcinoma. HCV is a small **(+)ssRNA** (Table 3). There are 6 genotypes. The virus cannot be grown in cell culture.

Epidemiology and transmission

1. HCV is prevalent in the Egypt. Incidence rates of 0.8/1,000 persons/year have been found in an area of Upper Egypt, where the prevalence was 9%, and 6.8/1,000 in the Nile Delta, where the prevalence was 24%. Sixty-seven percent of the incident infections were in persons younger than 20 years. Genotype 4 is widely distributed in the Middle East including Egypt.
2. The most efficient transmission of HCV is through large or repeated direct percutaneous exposures to contaminated blood (see table 5).
3. HCV is less efficiently transmitted by single small-dose percutaneous exposures e.g., exposure of healthcare workers to contaminated medical equipment and needlestick injuries.
4. Unlike HBV and HIV, HCV is less efficiently transmitted by mucosal exposures to blood or serum-derived fluids e.g., perinatally from infected mother to baby and sexual intercourse with an infected partner.
5. Receipt of infected blood products or organs was an important cause of HCV infection prior to 1992; before initiation of screening for HCV.
6. Environmental inapparent percutaneous exposures caused by cross-contamination from reused needles and syringes, multiple-use medication vials, and environmental surfaces.

7. Still a small proportion of cases (10%) exists with no identifiable route of transmission source.

Laboratory diagnosis

1. Detection of anti-HCV antibodies by ELISA, or the more specific RIBA test (recombinant immunoblotting assay). Seroconversion may take up to 6 months.
2. PCR for detection of viral RNA in blood. This is useful in:
 - a) Diagnosis of early cases before seroconversion.
 - b) Serologically confirmed cases to demonstrate active viral replication and, thus, the need for therapy.
 - c) Follow up the response to treatment.
 - d) Genotyping of HCV: The genotype is the strongest predictor of response to interferon and ribavirin therapy and thus is routinely determined during the evaluation of patients.

RIBA is more specific

100

Infection Prevention and Control

- No specific vaccine or immunoglobulin is available for immunoprophylaxis.
- Avoid exposure to bloodborne viruses (HCV, HBV, and HIV) through preventing exposure to patient's blood and other body fluids by practicing standard precautions (see chapter on infection control).
- In case of exposure you should promptly wash the injured site with soap and water or wash with saline in case of blood splash to the eyes vigorously. Post-exposure followup is recommended.

Treatment

A combination of alpha-interferon and antiviral chemotherapy (ribavirin) has been used and response depends upon the virus genotype. Genotypes 2 or 3 had response for therapy higher than that for genotype 1. Unfortunately subtype 4a is the most common in Egypt and has poor response to therapy.

↓ Most Common in Egypt

Hepatitis G virus/ GB virus type C (GBV-C)

GBV-C virus is a blood borne virus. Although most studies suggest that the virus is primarily hepatotropic, others contradict this finding instead suggesting that the virus is primarily lymphotropic and may replicate in CD4⁺ T cells.

(x) not for exam

Table (5): the infection source and transmission probabilities of the standard bloodborne viruses HBV, HIV and HCV

Infection source	Transmission probabilities		
	HBV	HIV	HCV
Household family contacts ^a	+	+	+
Institutionalized persons via close bodily contact ^b	+	+	+
Injection drug use (IDU) with shared needles	+	+	+
Blood product transfusions	+	+	+
Haemodialysis	+	+	+
Organ transplantation	+	+	+
Occupational exposures of healthcare workers in the healthcare settings to blood and serum derived body fluids: percutaneous (Needlestick), and permucosal (splash to eyes)	+	+	+
Other percutaneous exposures include ear piercing, tattooing, sharing razors and acupuncture	+	+	+
Intranasal cocaine	+	+	+
Sexual intercourse with an infected partner	+	+	±
Anal/oral sexual	+	+	ND
Oral (saliva) casual contact	ND	ND	ND
Passage of virus across the placenta during gestation ^c	±	+	ND
Perinatal from mother to child during birth ^c	+	+	±
Perinatal from mother via colostrum or milk ^c	ND	+	ND
Tears, sweat, urine, faeces, CSF, and synovial fluid	ND	ND	ND

+, Definite Transmission; ±, uncommon Transmission; ND. Not documented

^ainfection of children through minor skin breaks and mucous membranes

^binfection through minor skin breaks and mucous membranes

^cmodes of vertical transmission

Chapter 3

* enveloped +ve sense ssRNA
* Reverse transcriptase

RETROVIRUSES

Retroviruses are enveloped positive sense ssRNA viruses that contain the enzyme reverse transcriptase. This enzyme is responsible for a unique feature of replication not found in other viruses. Important members of *Retroviridae* family that affect humans are:

Genus	Virus species	Characteristics
<u>Lentivirus</u> ↳ <u>latency</u>	Human immunodeficiency viruses 1 and 2 (HIV-1 and HIV-2)	<u>Cytocidal</u> slow viruses; long incubation period, chronic disease with prolonged <u>clinical latency</u> with persistent viral replication and affection of the central nervous system. *** <u>because it affects cells</u>
<u>Deltaretrovirus</u>	Human T-lymphotropic virus 1 and 2 (HTLV-1 and HTLV-2)	Can <u>transform</u> cells <u>in vitro</u> but do not possess a specific oncogene.

clinical latency
↓
cells
↓
cells

Human Immunodeficiency Viruses

Since the initial description of HIV-1 in 1983 and HIV-2 in 1986, these two viruses have been identified as the primary cause of the acquired immunodeficiency syndrome (AIDS). HIV-1 is the major cause of AIDS worldwide, while AIDS caused by HIV-2 is much less severe, slower in progression, and limited mostly to west Africa. Both viruses replicate in CD4 T helper cells.

Structure: (Fig. 5)

The virus has a spherical shape with a diameter of 100 nm. It has a cylindrical (bar-shaped) core that contains the viral genome and is surrounded by an envelope.

* The viral envelope:

It is composed of a lipid membrane and contains 2 virus-specific glycoproteins: gp120 and gp41.

a) gp120 protrudes from the surface and is responsible for viral binding to host cell receptors.

b) gp41 is embedded in the envelope and mediates the fusion of the viral envelope with the cell membrane at the time of infection.

* The viral genome:

It consists of 2 identical copies of positive sense ssRNA, each of which has a copy of the virus nine genes: 3 typical retroviral genes (env, pol, gag) and 6 accessory genes.

c) env gene: encodes gp160, a precursor glycoprotein that is cleaved to form the 2 envelope glycoproteins gp120 and gp41. Rapid mutation in this gene results in many gp120 antigenic variants.

d) pol gene: encodes the enzymes reverse transcriptase, integrase and protease which participate in viral replication.

e) gag gene: encodes the core proteins, the most important of which is p24.

* virus slow, 11 جدة الكتاب

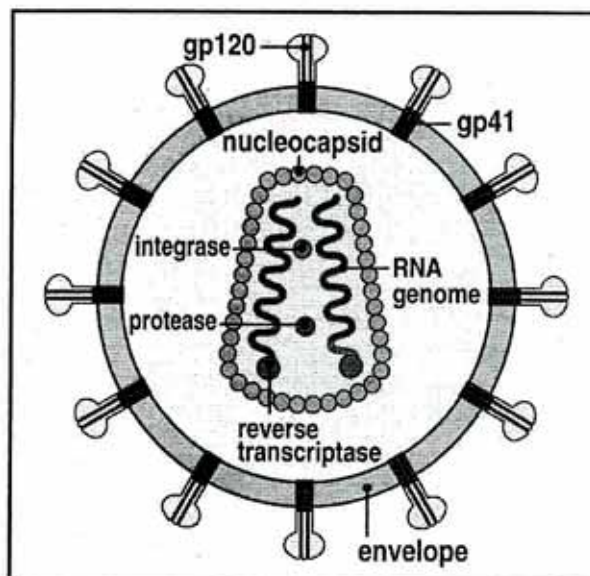


Fig. (5): Structure of HIV.

Blood
Semen
Vaginal secretion
Breast Milk

* Pathogenesis:

HIV transmission:

HIV transmission occurs when a person is exposed to body fluids infected with the virus, such as blood, semen, vaginal secretions and breast milk. The primary modes of HIV transmission are:

- 1) Sexual transmission: sexual contact (hetero- or homosexual) with an infected person.
- 2) Parenteral transmission: e.g. sharing needles or accidental pricking by a needle contaminated with infected blood. For this reason health care workers and drug addicts are among risk groups. Routine screening of blood for antibodies to HIV markedly reduced the risk of acquiring HIV from blood transfusions.
- 3) Vertical transmission: approximately 25% of HIV-infected pregnant women who are not treated during pregnancy, can transmit HIV to their infants during pregnancy, childbirth or through breast-feeding.

i) Blood transfusion

Cells infected by HIV:

- f) CD4 T helper cells → Main Target of virus
- Macrophages and monocytes.
- Dendritic cells
- Oligodendrocytes, astrocytes, neurons and glial cells
- Follicular dendritic cells (in lymph nodes)

APC's
Nerve cells



Except for the CD4 T lymphocytes, these cells are not necessarily destroyed by the virus; therefore they act as reservoir for further T cell infection.

Following sexual transmission, the dendritic cells in the genital mucosa bind to the virus and carry it from the site of infection to the lymph nodes where other cells (esp. T helper cells) become infected. Infection of CD4 T helper cells by HIV leads to their depletion by 2 main mechanisms:

- a) direct killing of infected cells by the virus as a result of viral replication.
- b) killing of infected CD4 T cells by CD8 cytotoxic lymphocytes which constitutes the main immune response to HIV infection.

CD4 T
CD8 T cytotoxic
Virus

The depletion of T helper cells leads to loss of cell-mediated immunity, increased susceptibility to infections and malignancies, and eventually death.

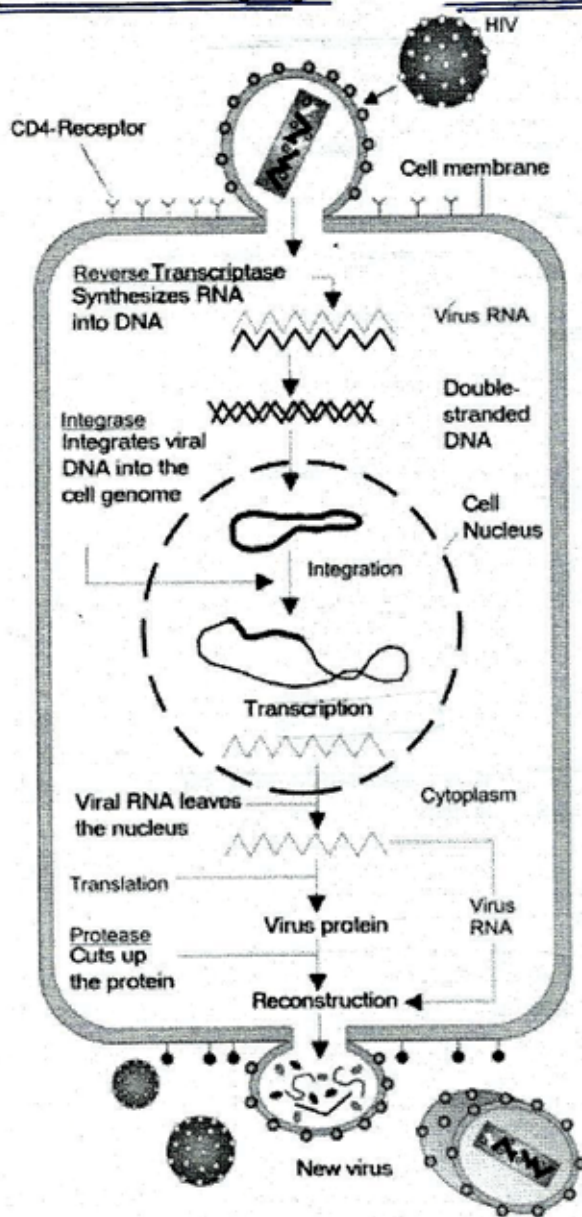


Fig. (6): Replication cycle of HIV.

Replication of HIV in infected cells:

The replication of HIV follows the typical retroviral cycle (Fig 6)

① Binding (attachment):

the viral gp120 bind to CD4 molecules followed by binding to a chemokine receptor on cell surface. Genetic deficiency of this chemokine receptor result in resistance to HIV infection

② Penetration:

the viral gp41 mediates fusion of viral envelope with cell membrane and virus enters the cell

③ Reverse Transcription:

the viral reverse ¹⁹transcriptase uses the viral RNA as template to form complementary DNA (cDNA)



4) Integration:

The newly formed HIV DNA moves to the cell nucleus, where viral integrase mediates its integration into the host cell DNA. Integrated HIV DNA is called *'provirus'.

5) Transcription:

HIV mRNA is transcribed from the proviral DNA.

6) Translation:

HIV mRNA is transported from the cell nucleus to the cytoplasm, where large viral polyproteins are made using HIV mRNA as a template.

7) Assembly and Release:

Assembly of new virus particles takes place at the membrane of the host cell giving an immature virion.

The HIV protease cleaves the polyproteins to produce the essential structural proteins and the 3 enzymes mentioned above. This cleavage process is necessary to give the mature, infectious virion, which is released by budding through the cell membrane.

Clinical findings:

The clinical picture of HIV infection can be divided into 3 stages:

A) Acute (early) stage:

- g) After an incubation period of 2-4 weeks HIV-infected individuals suffer an acute flu-like or infectious mononucleosis-like illness (**acute retroviral syndrome**). The most common symptoms are fever, maculopapular rash, oral ulcers, lymphadenopathy, sore throat and malaise.
- h) During this phase, the blood contains many viral particles that spread throughout the body (particularly lymphoid organs).
- i) The number of CD4 T cells is usually normal.
- j) This symptomatic phase lasts for 7-10 days, after which the cytotoxic T cells (the main immune response to HIV) and antibodies dramatically reduce HIV levels. This induced immune response succeeds in controlling, but not eliminating, the virus.

B) Latent (middle) stage:

- k) Acute infection is followed by an extended period of **clinical latency**.
- l) During this phase a large amount of HIV particles is being produced by lymph node cells but remains sequestered within the lymph nodes, i.e. during clinical latency the virus itself does not enter a latent stage. *7/10/12*
- m) The patient is usually asymptomatic and viraemia is low or absent.
- n) The duration of this period (which may extend up to 10 years) depends on a number of factors including the virus type, immune response and use of antiretroviral therapy.
- o) Clinical latency is actually attributed to the ability of HIV to evade the aggressive immune response through:
 - a) High rate of viral mutation (esp. env gene).
 - b) Integration of the virus in the chromosome of infected cells shielded from recognition by the immune system.
 - c) Downregulation of MHC I expression on infected cells by the virus preventing recognition of these cells by cytotoxic T cells.

Give reason for clinical latency



- d) Loss of CD4 T cell responses which leads to impaired functions of other cells of the immune system.

C) Immunodeficiency (late) stage:

- p) Viral replication and gradual depletion of CD4 T cells continue until finally, some years after initial infection, full-blown **AIDS** develops. This occurs when CD4 T cell count falls below 200/mm³ (normal count: 800-1200/mm³).
- q) The infected person becomes particularly susceptible to opportunistic infections and cancers characteristic of AIDS such as Kaposi's sarcoma and certain lymphomas. Patients suffer debilitating weight loss, diarrhea and neurologic manifestations.
- r) Infections are the major cause of death in AIDS patients. The most important opportunistic infections include:
- Fungal infections: *Pneumocystis jiroveci* (pneumonia)
Cryptococcus neoformans (meningitis)
Candida albicans (oral thrush)
 - Bacterial infections: *M. avium-intracellulare*
M. tuberculosis
 - Viral infections: CMV, HSV, VZV

Laboratory diagnosis:

1) Serologic diagnosis:

Diagnosis of HIV infection could be achieved by applying the **Standard testing protocol**. It consists of an initial antibody screen by ELISA (presumptive diagnosis), followed by confirmation of reactive results by Western blot test.

- s) **ELISA** is performed on patient's serum against a mixture of HIV1 and HIV2 antigens.
- a) If the test is non-reactive the patient is considered HIV-negative.
- b) If the test is reactive, ELISA is repeated in duplicate on a second serum sample.
- c) If result of both or any of the duplicate tests is reactive, the specimen is reported as repeatedly reactive and must be confirmed by the more specific Western blot for definitive diagnosis. (False reactive ELISA may occur in other conditions e.g. SLE and syphilis.)
- t) In the **Western blot** technique, individual HIV antigens embedded in nitrocellulose paper strip react with the patient's serum. If antibodies to HIV are present they will bind to the corresponding viral antigens (predominantly to gp41 and p24). Like ELISA, a detection enzyme is then added; a colour reaction indicates the presence of HIV antibodies in patient's serum. The final result is a series of shaded bands (Fig. 7).
- a) If no shaded bands appear the test is reported as negative.

	gp160		
SU	gp120		ENV
RT	p66 p51		POL
TM	gp41		ENV
IN	p32		POL
CA	p24		
			GAG
MA	p17		
IgG	control		

Fig. (7): HIV Western BLOT



- b) If 3 or more shaded bands appear there is solid evidence that antibodies to HIV are present.
- c) If 1 or 2 shaded bands appear the test is considered indeterminate, which might represent an incomplete antibody response to HIV antigens. Therefore, a second specimen should be taken at least 1 month later and retested. If the same indeterminate result appears after 6 months, a non-specific reaction NOT due to HIV disease is likely.

Only specimens that are **repeatedly reactive by ELISA** and **reactive by Western blot** are considered HIV positive and indicative of HIV infection.

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2011
Diagnosis by serologic tests cannot be made in the following cases where other diagnostic methods should be applied:

- a) During the acute stage as antibodies to HIV are not detectable except 3-4 weeks after infection ('window' period).
- b) Diagnosis of HIV in newborns born to infected mothers due to presence of passively acquired maternal IgG. (Commercial tests for HIV-specific IgM are not yet available.)

2) Molecular techniques for detection of viral nucleic acid:

These are sensitive and specific techniques.

- PCR can be used to detect proviral DNA within infected cells (qualitative test)
- RT PCR can be used to measure the amount of HIV RNA in plasma to determine viral load (quantitative test).

3) Antigen detection:

- p24 antigen can be detected by ELISA.
- Its presence indicates viral replication. It appears in patient's serum soon after infection, often disappears after antibodies develop and its reappearance late in infection indicates a poor prognosis.
- Detection of p24 antigen is now used for routine screening in blood centers to detect HIV during the 'window' period.

4) Virus isolation:

HIV can be grown in culture from clinical specimens, but this procedure is available only at a few medical centers.

5) CD4 count:

The diagnosis of AIDS is also established by the finding of a CD4 count of less than 200 cells/mm and a CD4/CD8 ratio of less than one.

Treatment:

No treatment is available that cures AIDS. A number of antiretroviral drugs have been developed that suppress HIV replication, thereby preventing the destruction of the immune system, but eradication of the virus still remains impossible. In addition, new problems related to drug toxicity and the occurrence of resistant mutants are emerging. Antiretroviral drugs target different stages in the replication cycle of HIV:

- 1) **Reverse transcription inhibitors** (e.g. the nucleoside analogues AZT and lamivudine). These interfere with synthesis of proviral DNA.

- 2) **Protease inhibitors** (e.g. ritonavir and indinavir). These interfere with cleavage of proviral polyproteins during budding resulting in the generation of immature non-infectious viral particles.

Protease inhibitors are the most potent antiretroviral drugs.

- 3) **Fusion inhibitors** (e.g. fuseon). These new anti-HIV drugs prevent binding or fusion of the viral envelope to lymphocytes.

Acute HIV infection is treated with a combination of 2 reverse transcription inhibitors and a protease inhibitor. This combination is known as **HAART** (Highly Active Antiretroviral Therapy). As patients may benefit from therapy at an early stage of infection, laboratory tests based on antigen detection or molecular methods should be applied to diagnose infections during the window period.

Monitoring anti-HIV therapy is done by determination of:

- a) **viral load** (by RT PCR)
- b) **CD4 count**: every 3-6 months during clinical stability, and more frequently if symptomatic disease occurs.

Prevention and Control:

- 1) **No vaccine** for human use is available. Trials are being carried out in animal models (monkeys) using a vaccine containing gp120. Antibodies against gp120 neutralize the infectivity of HIV; but the rapid appearance of gp120 genetic variants makes the production of an effective vaccine difficult.
- 2) Prevention consists of taking measures to **avoid exposure to the virus** :
 - In the community: through education and awareness of the public regarding transmission and avoidance.
 - In the healthcare setting : through applying the infection control practices of the **Standard Precautions** including proper handling and disposal of sharps (see Infection Control) and screening of blood before donation.
- 3) In case of exposure to the virus, **post-exposure chemoprophylaxis** should be started immediately and continued for 2-4 weeks (see Infection Control).
- 4) Proper **disinfection of surfaces** contaminated by blood or body fluid spills should be done by using disinfectants such as hypochlorite solution (1% available chlorine for 10 min.)
- 5) To prevent **vertical transmission** screening of pregnant women for HIV should be done especially among high risk populations. In case of infected women the following measures are to be taken:
 - Treatment of infected mothers during pregnancy.
 - Delivery by cesarean section rather than vaginal delivery.
 - Treatment of neonates by effective antiretroviral drugs.
 - No breast feeding.

Chapter 4

PICORNAVIRUSES

Picornaviridae family represents the smallest (Pico) RNA viruses (27-30 nm in diameter). Important genera include:

1. **Enterovirus:** members are acid resistant and infect man orally. Table 6 shows the different groups and serotypes and major diseases they produce.

Table (6): *Enterovirus* groups and serotypes and major diseases.

Group and (serotypes)	Major disease
Poliovirus: 3 antigenic types (1-3)	1. Paralytic poliomyelitis 2. Aseptic meningitis
Coxsackieviruses A (1-22, 24)	1. Aseptic meningitis 2. Herpangina: is an acute painful febrile pharyngitis, characterized by vesicles on the fauces and tongue which rapidly ulcerate. 3. Acute haemorrhagic conjunctivitis.
Coxsackieviruses B (1-6)	1. Aseptic meningitis 2. Fatal neonatal myocarditis 3. Pleurodynia: characterized by stabbing pain in the muscles of the chest and epigastrium. 4. Myo- or pericarditis 5. Juvenile diabetes mellitus
Echoviruses (Enteric Cytopathogenic Human Orphan Viruses) (1-9, 11-27, 29-34)	1. Aseptic meningitis 2. Febrile illness with or without rash
Enteroviruses (types 68 -71)	- 68 → Bronchiolitis or pneumonia in children. - 70 → Acute haemorrhagic conjunctivitis. - 71 → Aseptic meningitis, encephalitis and paralysis.

2. **Hepatovirus (HAV).**
3. **Rhinovirus:** > 100 antigenic types.
4. **Aphthovirus:** The causative agent of [الحمى القلاعية] **foot-and-mouth disease** that occurs primarily in cattle and sheep, and can be transmitted to humans by contact or ingestion of infected meat.

Poliovirus

Properties of the virus

1. There are three antigenic types (1, 2 and 3).
2. They are stable at acidic pH, resistant to ether, deoxycholate and other detergents.
3. They are inactivated by pasteurization and chlorine.
4. Primates, e.g. human and monkeys are the only susceptible hosts.
5. The virus can be grown in cell line cultures derived from human tissues, monkey kidney, testis or muscles giving a characteristic CPE.



Poliomyelitis

Poliomyelitis is an acute infection that involves the gastrointestinal tract and, occasionally, the central nervous system. Humans are the only reservoir of poliovirus infection.

Pathogenesis and clinical features

Infection occurs by ingestion of food and drinks contaminated by stools from cases or carriers (i.e. faeco-oral), the incubation period is 7-14 days. The virus multiplies initially in the lymphoid tissues of the tonsils and Peyer's patches in the small intestine then spread to the local lymph nodes (cervical and mesenteric lymph nodes). Then, the virus may invade the blood (viraemia) and reach the CNS. The paralytic effect of poliovirus results from damage of the motor neurons of the anterior horn of the spinal cord or the bulbar region of the brain stem. Most of the infections are subclinical, only 1% of the infections result in clinical illness. The infection may occur at various stages as follows:

1. Inapparent infection: The virus multiplies in the intestine without invading the blood stream or CNS. It is excreted in faeces. At this stage, protective immunity develops against re-infection with the same antigenic type.

2. Abortive infection: It is the most common form. The virus invades the blood stream (viraemia) but not the CNS. Patients present with mild symptoms as fever, malaise, headache, nausea and vomiting.

3. Aseptic meningitis (non-paralytic poliomyelitis): The virus reaches the CNS. The manifestations are headache, neck stiffness and backpain. Rapid and complete recovery is usual but may progress to paralysis.

4. Paralytic poliomyelitis: Flaccid paralysis develops in 0.1-1% of infections especially in children. Infections of the nerve cells in the brain stem will cause bulbar paralysis and death from respiratory failure.

Outcome

1. Solid immunity develops to the same antigenic type.
2. Faecal carriage of the virus usually occurs for several months.

Treatment

There is no antiviral drugs for the treatment of poliovirus infection.

Prevention and control

1. Improvement of systems of hygiene and sanitation to stop virus transmission.
2. Immunization

A- Active immunization: There are two effective vaccines containing the three antigenic types. The vaccines used for active immunization are Sabin which is a live oral poliovirus vaccine (OPV) and Salk which is inactivated poliovirus vaccine (IPV). The vaccine should be given to infants in three doses at 2, 4 and 6 months. Booster doses are given at 18 months and at school entry. Table (7) summarizes differences between both vaccines.



Table (7): Differences between Sabin vaccine and Salk vaccine

	Sabin vaccine Oral poliovaccine (OPV)	Salk vaccine Inactivated poliovaccine (IPV)
Type	<u>Living attenuated virus</u>	Formalin inactivated (killed) whole poliovirus
Route of administration	oral	Intramuscular
Protective immunity	- Local intestinal immunity by secretory IgA against reinfections. It breaks transmission in the population. - Systemic immunity by humoral IgG and IgM antibodies → prevent invasion of CNS.	- <u>NO</u> Local immunity. So virus is still able to multiply in the gut. - Systemic immunity <u>ONLY</u>.
Dissemination of vaccine virus in the community	Vaccine virus passes in the stools and can be transmitted to non immunized children.	NO
Use in pregnancy	contraindicated	Safe
Use in immunosuppressed individuals and their household contacts	contraindicated <i>because May cause disease by virus itself</i>	Safe
Potential limiting factors	1. <u>Block of immunization due to Interference by other enterovirus infections at the time of vaccination.</u> 2. Rare occurrence of vaccine associated poliomyelitis due to <u>mutation of the virus to the wild type.</u> 3. Loss of potency due to improper storage (properly stored at 4° C)	<u>None</u> of these.
Allergy (Minor local reactions)	NO	YES*

* **Allergy** because IPV contains trace amounts of streptomycin, polymyxin B and neomycin, hypersensitivity reactions can occur among people sensitive to them.

N.B. Breast-feeding is not a contraindication to immunization against poliovirus.

B- Passive immunization: Gamma globulin is effective only if given early to contacts. It is of no value if given after development of clinical symptoms.



C.R. Repeated infection

The rhinovirus group derives its name from the predominant site of replication (the nose). The rhinovirus group is the commonest cause of the acute respiratory illness known as the **common cold**. Over 100 serotypes of rhinoviruses are currently recognized.

Pathogenesis and clinical significance

- Transmission is through droplet and hand-to-hand contact followed by self-inoculation of virus into the nose or conjunctiva.
- Incubation period is 2 - 3 days.
- The virus multiplies locally without blood invasion.
- Peak of virus shedding coincides with acute rhinitis, and virus becomes usually undetectable after 4 or 5 days.
- Type specific immunity that correlates best with the local production of IgA.
- Secondary bacterial infection may cause otitis media, sinusitis, bronchitis or bronchopneumonia especially in children.

Repeated infection of common cold could be explained by:

1. Multiplicity of antigenic types (over 100).
2. Being a superficial infection, serum antibodies plays no significant role in immunity.
3. Immunity is mainly superficial by IgA and interferon (short term immunity).

Vaccine production is not practical.

Chapter 5

ORTHOMYXOVIRUSES AND PARAMYXOVIRUSES

Distinction of the family *Orthomyxoviridae* from *Paramyxoviridae* is in table (8).

Table (8): *Orthomyxoviridae* and *Paramyxoviridae*

Characteristic	<i>Orthomyxoviridae</i>	<i>Paramyxoviridae</i>
Diseases caused in humans	Influenza types A, B, C	Parainfluenza virus 1-4 infections, <u>mumps</u> , <u>measles</u> , <u>respiratory syncytial disease</u> .
Particle size	Smaller (80 – 120 nm)	Larger (150 nm or more)
Transcription of viral RNA	Host cell nucleus	Host cell cytoplasm
Genome	Segmented (-)sense RNA	Non-segmented (-)sense RNA
Gene reassortment	Frequent	Rare
Rate of antigenic change	High	Low

Influenza Viruses

The members of the family *Orthomyxoviridae* are all influenza viruses. They have the following characters:

- They replicate in mucous membranes of the upper and lower respiratory tracts.
- They contain a **segmented single-stranded RNA** genome.
- They are enclosed in a lipid envelope and a layer of glycoprotein spikes known as haemagglutinin (HA) and neuraminidase (NA) which are the major antigenic determinants.
- Influenza viruses, types A, B, C cause influenza. Influenza occurs in epidemics and pandemics.

Classification, antigenic types and host range

- *Influenza viruses* are divided into types A, B, and C on the basis of the nucleoprotein antigen.
- In types A and B, the HA and NA antigens undergo genetic variation, which is the basis for the emergence of new strains; type C is antigenically stable.
- **Influenza A virus** only is further classified into **subtypes** based upon HA and NA antigens. 16 HA subtypes and 9 NA subtypes are now recognized circulating in birds, humans, swine and horses. A new subtype usually initiates panzootic in birds or pandemic in humans due to lack of pre-existing protective immunity to the new virus. Currently the most famous subtypes are:
A (H1N1) circulating in humans and is causing the 2009 influenza pandemic (swine flu),
A (H5N1) circulating in birds causing avian flu and infecting humans who closely handle infected birds.
- **Influenza B viruses** infect mammals only, but generally not as severe as type A.

Morphology and structure (Fig. 8)

- Influenza viruses are spherical, although filamentous forms may also occur.
- **Genome:** Consists of s/s (-) sense RNA in 8 segments (7 in Influenza C).
- Segments with nucleoprotein form a nucleocapsid with helical symmetry.
- The nucleocapsid is enclosed in an envelope consisting of a lipid bilayer and two surface glycoproteins, a haemagglutinin and a neuraminidase.

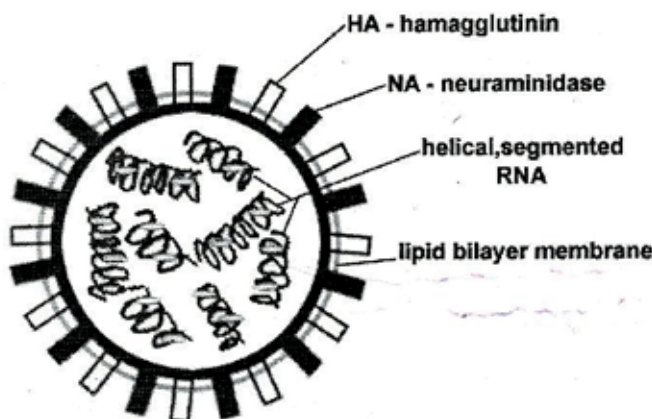


Fig. (8): Structure of Influenza virus.

Gene reassortment

Because the influenza virus genome is segmented, genetic reassortment can occur when a host cell is infected simultaneously with viruses of two different parent strains. If a cell is infected with two strains of type A virus, for example, some of the progeny virions will contain a mixture of genome segments from the two strains. This process of genetic reassortment probably accounts for the periodic appearance of the novel type A strains that cause influenza pandemics (see below).

Antigenic change of the influenza viruses and its impact on influenza epidemics and vaccine preparation

Influenza epidemics are of two types: (1) Yearly epidemics are caused by both type A and type B viruses and (2) The rare severe influenza pandemics are always caused by type A virus. Two different mechanisms of antigenic changes are responsible for producing the strains that cause these two types of epidemic:

1. **Antigenic shift:** A major change in one or both of surface antigens. It yields an antigen showing no serologic relationship with the strains prevailing at the time of epidemics. It occurs in type A virus only and produces strains responsible for influenza pandemics. The completely new antigens that appear during antigenic shift are acquired by gene reassortment. The donor of the new antigens is probably an animal influenza virus.

2. **Antigenic drift:** Repeated minor antigenic changes which generate strains that retain a degree of serologic relationship with the currently prevailing strain. Antigenic drift occurs in both type A and type B and is responsible for strains that cause yearly influenza epidemics. Antigenic drift represents selection for naturally occurring variants under the pressure of population vaccination and immunity.



Pathogenesis and clinical picture

- It is transmitted by droplets and contact with contaminated surfaces.
- Virus NA may liquefy mucus and help spread through the respiratory tract.
- Incubation period is 1-2 days.
- Infection of mucosal cells results in cellular destruction and desquamation of superficial mucosa. Viraemia is rare.
- The patient suffers from chills, malaise, fever, muscle pain (myalgia) and respiratory symptoms as rhinitis, pharyngitis ... etc..
- Pure viral pneumonia may occur but more commonly bacterial pneumonia associated with influenza (caused by secondary infection by *S. pneumoniae*, *S. aureus* and *H. influenzae*).

Prevention

1. Immunoprophylaxis: Influenza vaccines:

- a) Inactivated (Killed) influenza virus vaccines have been used to prevent influenza. A given vaccine contains the strains of types A and B viruses that are judged most likely to produce epidemics during the following winter. The intramuscular route is recommended. Two doses administered at least one month apart are required for previously unvaccinated infants and children <9 years of age. In adults, only one dose is administered. A booster is not recommended.
- b) Live attenuated vaccines given intranasally are being developed as alternatives to inactivated vaccine.

2. Chemoprophylaxis:

- **Amantadine and rimantadine** drugs effectively prevent infection and illness caused by type A, but not by type B, viruses.
- **Neuraminidase inhibitors (oseltamivir):** are active against influenza A and B.

Treatment:

- Amantadine and rimantadine are effective only against type A.
- The neuraminidase inhibitors zanamivir and oseltamivir are active against types A and B.

The following viruses belong to the Paramyxoviridae family:

Parainfluenza Viruses

- They cause severe lower respiratory tract disease particularly in infants and young children.
- After an incubation period of 2-6 days, the disease manifests as mild fever, rhinitis and bronchitis.

Spasmodic laryngotracheitis (croup) and pneumonia may occur in severe cases.



Mumps

by droplets

- Mumps is an acute contagious disease of children characterized by nonsuppurative enlargement of one or both parotid glands (parotitis).
- Mumps virus mostly causes a mild childhood disease, but in adults it may be complicated with **meningitis** or **orchitis**.
- At least 1/3 of infections are asymptomatic.

Pathogenesis and clinical findings

- Man is the only host for mumps virus.
- Transmission is by droplets.
- The virus replicates locally in the respiratory mucosa and regional lymph nodes, followed by viraemia and localization of the virus in salivary glands especially the parotid glands. The virus may also disseminate into testes, ovaries, pancreas and brain.
- After an incubation period of 2-3 weeks, the disease manifests as fever and painful swelling of the parotid glands.

Prevention and control

1. Living attenuated vaccine for children over 1 year. It is given in a single IM dose.
2. Mumps vaccine is available in combination with 2 other living attenuated virus vaccines, measles and rubella (**MMR vaccine**). It is given in two doses, the first at 15 months and the second at school age (4-6 years).

Immunity is life-lasting immunity after a single infection. This is because:

- Only one stable antigenic type exists.
- There is a stage of viraemia, so circulating virus neutralizing antibodies (IgM and IgG) are protective.

Measles (Rubeola) Virus

by Aerosol

- Measles is an acute highly infectious disease of children and non-immune adults.
- The virus has only one stable antigenic type.

Pathogenesis and clinical findings

- Man is the only natural host for the virus.
- Transmission is by aerosol.
- Incubation period is 8-12 days.
- The virus replicates locally in the mucosa and regional lymph nodes of the upper respiratory tract followed by viraemia and localization of virus in skin and mucous membranes.
- After a prodrome of 2-4 days, a viraemic phase starts that manifests by high fever, sneezing, coughing, eye pains and as "Koplik's spots" on the inside of the cheeks. The eruptive phase follows and is characterized by maculopapular pink skin rash mainly on the trunk region.

- Complications may occur in debilitated children as pneumonia, otitis media, and post-measles encephalitis.

Treatment

- No specific antiviral drug is effective.
- Early administration of human globulins may be effective in modifying the disease.
- Bacterial superinfections are to be treated by antibiotics.

Prophylaxis

Active immunization by a living attenuated vaccine (MMR) as above.

Passive immunization: Human globulins are given to protect susceptible contacts.

Immunity: Life-long immunity is gained after a single infection. This is due to:

- Measles virus is of only one stable antigenic type.
- There is a stage of viraemia, so circulating antibodies are protective.

Respiratory Syncytial Virus (RSV)

- RSV is an important cause of lower respiratory tract disease in infants and young children, under one year of age.
- No viraemia.
- Most cases are asymptomatic.
- The disease ranges from common cold-like illness in adults to febrile bronchiolitis, bronchitis, and pneumonia in infants and young children.

Diagnosis

Antigen detection in respiratory secretions

Treatment

Ribavirin aerosol.

Prophylaxis

No specific prophylaxis is available.



Chapter 6

RUBELLA (German Measles)



Properties of the virus

- Rubella virus is a member of the Rubiviruses of the *Togaviridae* family.
- It is a single stranded (ss) RNA virus.
- It is of only one antigenic type.

Pathogenesis and clinical findings

- Man is the only natural host of rubella virus.
- Transmission is by droplets.
- It mainly affects children.
- The virus multiplies in the respiratory mucosa. Viraemia develops followed by the appearance of maculopapular rash and cervical lymphadenopathy.
- The rash starts on the face and spreads downwards for 2-3 days then disappears.
- 25% of the cases are asymptomatic.

Infection during pregnancy

Although rubella is a mild self-limiting disease, if it is acquired during the first trimester of pregnancy it results in serious complications. There may be abortion, intrauterine foetal death, or congenital malformations of the baby as mental retardation, deafness, heart anomalies, microcephaly, cataract and blindness. This is called congenital rubella syndrome.

TC 2011

Laboratory diagnosis

- It is not usually necessary except in pregnant females. Detection of rubella antibodies by ELISA, either IgM or a rising titre of IgG, denotes recent infection and a therapeutic abortion may be indicated.
- Detection of IgM antirubella antibodies in a newborn confirms intrauterine infection.

Prevention and control

- A live attenuated vaccine usually given in combination with that of measles and mumps (MMR) at the age of 15 months, and a booster dose is given at the school age.
- As immunity may wane in individuals vaccinated at childhood, reimmunization is recommended for prepubertal girls and mothers in the immediate postpartum period.

Rubella vaccine virus can cross the placenta but is not teratogenic. Still, it is advisable to avoid vaccination during pregnancy. Vaccine should delay conception for at least 3 months.





Chapter 7

CORONAVIRUSES

- Large, enveloped, positive sense stranded RNA viruses. They have the largest RNA genome.
- *Coronaviridae* family was distinguished on basis of its distinctive morphology and received the name *corona* because of the crown like appearance of the surface projections.
- The **human coronaviruses (CoVs)** are responsible for about 30% of mild upper respiratory tract illnesses, (**common cold**). Recently, CoVs have received the newly emerged SARS-CoV which causes the severe acute respiratory syndrome (SARS) that has been reported in Asia, North America, and Europe.

SARS transmission

Droplets and contact of the skin or fomites that are contaminated with infectious droplets and then touching eyes, nose or mouth.

Clinical aspects of SARS

- After an **incubation period** of 2-10 days, **onset** of the disease is characterized by **fever**, chills/rigors, headache, myalgia and malaise.
- Respiratory symptoms often begin 3-7 days after symptom onset and peak in the second week.
- Diarrhoea has been a prominent feature of early illness in some cases

Chapter 8

REOVIRUSES, CALICIVIRUSES AND ASTROVIRUSES

Reoviridae Rotavirus

* wheel appearance
* only virus dsRNA
double str
coat
segmented



Reoviruses are medium sized viruses with a segmented dsRNA genome. The family includes the human rotaviruses, the most important cause* of **infantile gastroenteritis** around the world.

Rotavirus genome is surrounded with a double-layered protein coat giving the wheel appearance by electron microscope (Rota = wheel). It is transmitted via faeco-oral route. Incubation period is 1-4 days.

Laboratory diagnosis

1. Examination of stools by electron microscope to detect the wheel-like viral particles.
2. Detection of viral antigen in stools by serological tests.
3. Using rotavirus specific nucleic acid probe to detect virus particles in stools (dot hybridization).
4. Demonstrating rising titres of the serum antibodies by ELISA.

Treatment

By restoring fluid and electrolyte balance.

Prophylaxis

- Hygienic measures to control faeco-oral infections.
- Rotavirus vaccine: Living attenuated vaccine is given orally in 3 doses at 2, 4 and 6 months of age.

Caliciviridae Norwalk Viruses



The best known of the human caliciviruses is **Norwalk virus**, which is the most important cause of **epidemic viral (nonbacterial) gastroenteritis in school children and adults**. Norwalk virus was first discovered during an epidemic of gastroenteritis that occurred in Norwalk, Ohio, in 1968.

Characteristic features

- Caliciviruses are small (27-nm), round nonenveloped positive sense ssRNA viruses.
- They have a single structural protein.



- They have cup-shaped depressions on their capsid surface (hence their name, which is derived from calyx). These indentations give the virion a 'Star of David' appearance in the electron microscope.
- Human caliciviruses have not been cultivated *in vitro*.

Epidemiology

About half of the outbreaks of acute infectious nonbacterial gastroenteritis are due to Norwalk virus. The disease occurs worldwide and is common in older children and adults. Outbreaks occur in such settings as camps, restaurants, cruise ships and schools. Outbreaks are associated with contaminated food, water, raw or undercooked shellfish, particularly oysters. Norwalk virus has a low infectious dose (10 virus particles) and relative stability in the environment (resists chlorine).

Pathogenesis

The viruses can be transmitted from person to person by the faeco-oral route. Viruses grow in the small intestine, causing transient lesions of intestinal mucosa, and are shed in faeces.

Diagnosis

The diagnosis is suggested by acute gastroenteritis in an outbreak setting. It may be confirmed by antigen detection or observation of an antibody rise.

Control

No specific antiviral therapy or vaccine is available for Norwalk or other human calicivirus gastroenteritis. Hand washing and careful monitoring of water purification are the most important measures in the control of infection.

Astroviruses

pointed star shaped

Astroviruses are about 30 nm diameter and contain a positive-sense ssRNA. They exhibit a distinctive morphology in the electron microscope due to presence of pointed star shapes on the particles.

Astroviruses cause sporadic gastroenteritis in infants, children, elderly and immunocompromised people. Astroviruses are transmitted from person to person by the faeco-oral route through contaminated food, water, person-to-person contact, or contaminated surfaces.

RHABDOVIRIDAE

Rabies Virus

فردوس الابرار
الكلاب

Bullet shape

Rabies virus is bullet shaped of 75 by 180 nm. It contains helically coiled nucleoprotein. The virus is surrounded by an envelope with protruding spikes, composed of a single glycoprotein, that are needed for virus attachment to the host cells. The genome is a negative sense ssRNA with an RNA dependent RNA polymerase.

There is only one serotype. However, two biotypes have been described:

*Compare bet. strut & fixed

- Propagated in nature (wild).
- All laboratory animals are susceptible via peripheral or central route of infections.
- The incubation period is variable (20-90 days) allowing formation of intracytoplasmic inclusion bodies, known as Negri bodies, in the brain of infected animals.

- A mild virus that is attenuated in the laboratory by repeated passage in animals or cell culture.
- It causes encephalitis in laboratory animal by central (intracerebral) inoculation only.
- The incubation period is fixed (4-6 days) and there are no inclusion bodies.

The fixed virus type is used in preparation of the different rabies vaccines after being inactivated by the beta-propiolactone.

Rabies

Rabies is an acute fatal encephalomyelitis. It is a zoonotic viral disease which infects domestic and wild animals.

الحفاش لا يمتد له
لكن ينقله (٢٢)

1. Dogs are the main reservoir and transmitter of rabies to humans.
2. Wild carnivores, e.g. foxes and wolves.
3. Bat species are hosts, transmitters, or victims of rabies.

Other animals, e.g. cattle, camels, and horses are susceptible and do not transmit the disease. They are victims of rabies and usually epidemiologic dead-ends.



Transmission

The virus is present in the saliva of infected animals (e.g. dogs, cats ... etc). Rabies is almost always transmitted by rabid animal bites that inoculate the virus into wounds. Transmission by non-bite exposures is very rare, e.g. recipients of transplanted corneas from infected humans.

Pathogenesis

The virus replicates retrograde only ^{→ *Leptospirosis} ^{*Tetanus} at the site of infection. It travels from the bite site along the peripheral nerves to the CNS (no viraemia). The virus multiplies in nerve cells causing their damage and formation of "Negri bodies".

Incubation period

2 weeks-3 months; it is shorter in children and with bites in the head and neck, and longer when bites are in the lower limbs.

N.B.: The long incubation period allows active immunization, given after bite, to be effective in prevention of the disease.

Clinical manifestations

Once symptoms of the disease develop, rabies is fatal to both animals and humans. Three distinct phases of the disease are described. Each phase has a different set of symptoms.

1. The prodromal phase: The initial symptoms of rabies are often mild fever and pain or paraesthesia at the wound site. As the virus spreads in the central nervous system, progressive encephalitis develops.

2. Furious rabies = hyperactivity: The person or animal becomes extremely irritable and aggressive. Furious rabies is rapidly fatal brainstem encephalitis characterized by hydrophobia or aerophobia, hyperactivity and fluctuating consciousness. Strange behaviour and a lack of focal neurological signs are typical features.

3. Dumb rabies = paralytic rabies: Ascending flaccid paralysis with pain in the affected muscles will precede death from bulbar and respiratory paralysis.

Laboratory diagnosis

A) Diagnosis of rabies infection: ^{G.R.} No tests are currently available to diagnose rabies infection in humans before the onset of clinical disease. Furthermore, since the virus resides intracellularly, probably within myocytes or neurons at the site of the bite, it is difficult to detect and does not stimulate antibodies until late in the infection.

B) Diagnosis of rabies disease

Rabies diagnosis in humans: Early diagnosis is difficult. Several tests are necessary to diagnose rabies ante-mortem (before death) in humans; no single test is sufficient:

1. Detection of rabies virus antibodies in CSF by immunofluorescence: The CSF usually shows high titre of neutralizing antibody. The presence of antibody in the serum does not differentiate between natural infection and vaccination. The CSF usually also shows high protein level.

2. **Detection of rabies antigen**, by direct immunofluorescence, in the nerves surrounding the hair follicle in the nape of the neck.
3. **Isolation of rabies virus** from saliva, CSF, or CNS tissues by intracerebral inoculation in mice.

Rabies diagnosis in animals

1. **Demonstration of Negri bodies** in the hippocampus and cerebellum by direct immunofluorescence.
2. **Detection of rabies virus nucleic acid by RT-PCR.**
3. **Electron microscopy:** Rabies virus is seen as bullet-shaped particles.

Management of rabies

There is no cure for the clinical disease. Antiviral agents, interferon and rabies immune globulin failed to treat human cases. Fortunately, rabies infection can be aborted by using preventive therapy in the form of post-exposure prophylaxis (PEP). PEP consists of the combination of local wound treatment, rabies immune globulin (RIG) and rabies vaccine (Table 9).

Post-exposure prophylaxis is indicated based on risk of exposure:

1. All unprovoked animal attacks are considered high risk of rabies.
2. Bites or scratches from high-risk animals, such as bats, foxes and non-domestic dogs should be treated immediately. Contact with bats may result in viral transmission without obvious injuries.
3. All bite exposures with anticipated short incubation periods as in children or near to the CNS as in bites in the head and neck and in extensive bite wounds: PEP must be initiated immediately without waiting until signs of rabies or lab results are detected in a captured animal. If proved later on that the animal is not rabid discontinue the remaining vaccine doses.
4. If a bite or mucous membrane exposure was inflicted by a domestic animal with known vaccination status, and if the animal can be quarantined for 10 days, PEP can be withheld. If the animal survived this period, rabies is excluded. If signs of rabies occurred, the animal should be sacrificed and tested for rabies.
5. Exposures involving small rodents (e.g. rats, mice, rabbits, guinea pigs and hamsters) do not require treatment. These animals can be infected with rabies virus in the laboratory but have never been associated with transmission to humans.
6. No human-to-human transmission has been documented except among recipients of corneas. Anyone exposed to CSF, saliva, or mucous membrane of a person suspected of having rabies, should receive complete prophylaxis.

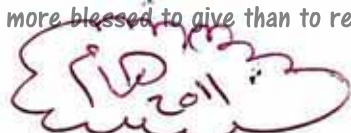


Table (9): Rabies post-exposure prophylaxis schedule

Vaccination Status	Management	Regimen*
Not previously vaccinated	Emergency care	PEP should always begin with immediate and thorough washing of all bite wounds and scratches with soap or a detergent (<u>2% benzalkonium chloride</u>) and water. Irrigate with a virucidal agent such as povidone-iodine solution. Tetanus prophylaxis and measures to control bacterial infection also should be administered as indicated.
	<u>RIG</u>	Administer <u>20 IU/kg body weight</u> . If anatomically feasible, the <u>full dose should be infiltrated around the wound(s)</u> and any remaining volume should be administered IM at an anatomical site distant from vaccine administration. RIG should not be administered in the same syringe as vaccine. Because RIG might partially suppress active production of antibody, no more than the recommended dose should be given.
	<u>Vaccine</u>	<u>Five IM doses (1.0 mL each in deltoid area)**</u> of HDCV, RVA, or PCEC vaccine, one each on days 0,3,7,14, and 28.
Previously vaccinated	Emergency care	PEP should always begin with immediate cleansing of all wounds as mentioned above.
	RIG	RIG should not be administered.
	Vaccine	<u>Two IM doses (1.0 mL each in deltoid area)**</u> of HDCV, RVA, or PCEC vaccine, one immediately and one 3 days later.

*These regimens are applicable for all age groups, including children.

** The deltoid area is the only acceptable site of vaccination for adults and older children. For younger children, the outer aspect of the thigh may be used. Vaccine should never be administered in the gluteal area.

Rabies vaccines

A) Crude rabies vaccines based on attenuated virus from nerve tissue:

1. **Pasteur** vaccine prepared from attenuated virus from desiccated nerve tissue.
2. **Semple** vaccine prepared from inactivated vaccines produced in sheep or goat brains.

23 injections

Can Cause type III Hypersensitivity

Pasteur sample

Unfortunately, the majority of post-exposure immunizations against rabies are still performed with these vaccines of crude nerve tissue origin. They may be associated with serious adverse events such as meningoencephalitis. As regard protective potency these vaccines are inferior to cell-derived vaccines. A complete post-exposure treatment using nerve tissue vaccines involves a prolonged and painful immunization course of up to 23 injections. Obviously, these vaccines are not recommended for pre-exposure immunization.

±

4-5 IM doses

0, 3, 7, 14, 28

B) Cell-derived vaccines:

1. **Human diploid cell rabies vaccine (HDCV):** is prepared on human diploid cell culture, concentrated by ultrafiltration, and inactivated with beta-propiolactone. It is regarded as the gold standard for rabies vaccines.
2. **Rabies vaccine adsorbed (RVA):** is prepared from rabies virus adapted to fetal rhesus lung diploid cell culture. The vaccine virus is inactivated with beta-propiolactone and concentrated by adsorption to aluminum-phosphate.
3. **Purified chick embryo cell vaccine (PCEC):** is prepared in primary cultures of chicken fibroblasts. The virus is inactivated with beta-propiolactone.
4. **Purified Vero cell rabies vaccine contains the Wistar strain of the virus.**

no cross allergy

There are no contraindications to any of these vaccines being used for post-exposure treatment. Should an allergic reaction occur, the modern vaccines of different cell substrate origin may replace each other. Pregnancy is not a contraindication to post-exposure treatment. Although associated with mild and transient reactions, all the cell-derived rabies vaccines are considered safe.

Passive immunization

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Passive immunization with **human rabies immune globulin (HRIG)** provides immediate protection. 20 IU/Kg is given, with as much of the dose as possible infiltrated in and around the wound and the rest is given deeply intramuscular in the gluteal region.

Individuals under immunosuppressive therapy should receive HRIG instead of the vaccine.

Pre-exposure prophylaxis

It is given to high risk individuals e.g. animal handlers, laboratory workers, veterinarians and travellers to areas where rabies is common. Rabies vaccine (HDCV or RVA) is given in 3 doses at days 0, 7 and 28 with boosters every 2-3 years.

@enveloped
RNA virus
arthropode



Chapter 10

ARBOVIRUSES AND ROBOVIRUSES

Rodent transmission
no arthropods involved

The name "arbo" is derived from (arthropod-borne) and is used to define viruses transmitted between vertebrate hosts by blood sucking arthropods. Arboviruses replicate in both insect (without obvious disease) and mammalian cells. Common features include the fact that these are **enveloped viruses with an RNA genome**. The association of an enveloped structure with transmission by arthropods has significance. Enveloped viruses readily lose infectivity and are spread most efficiently by either intimate contact or insect bite. Various animals, rodents and birds are reservoirs of infection.

Give reason
role of
rodent
&
arthropods
significant
for virus

A new group of serotypes are not transmitted by arthropods but are maintained within the rodent reservoir which may transmit infection directly to human without participation of arthropods. These are named **roboviruses**.

Humans are frequently an accidental host with the natural cycle most commonly involving wild vertebrates and blood-sucking insects. Therefore, human pathogens of the arboviruses are **zoonotic** since humans do not play important role in the transmission cycle except in urban yellow fever and dengue fever.

Classification

There are about 100 human pathogens of this large group that are included in *Togaviridae*, *Flaviviridae*, *Reoviridae* and *Bunyaviridae* families (Table 10).

Rubella virus is a member of *Togaviridae* but not transmitted by arthropods.

Hepatitis C virus is now classified in the *Hepacivirus* genus within *Flaviviridae* family.

Human Arbovirus Infections

1. Encephalitis

Encephalitis is caused by members in the *Togaviridae* and *Flaviviridae* families. Arbovirus encephalitis occurs in distinct geographic distribution and vector patterns. In Egypt, two viruses exist: West Nile virus and Sindbis virus.

Pathogenesis and clinical findings

Infection is acquired from the bite of infected mosquito. The virus multiplies in the reticuloendothelial cells, released into the blood (viraemia) which coincides with the onset of fever, the virus then reaches the CNS causing meningitis or meningoencephalitis. Inapparent infections are common.

Diagnosis

1. Virus isolation from blood (early in the febrile illness), from CSF or from brain tissue at autopsy of fatal cases. The viruses can be isolated by intracerebral inoculation of suckling mice.
2. Serological tests: Detection of rising titre of specific antibodies by CF, HI or ELISA tests.

17D vaccine
10 years
active

2. Yellow Fever

by yellow fever virus
flavi virus

Yellow fever is an acute, febrile, haemorrhagic disease caused by yellow fever virus which is a member of *Flaviviridae* family.

Pathogenesis and clinical findings

The virus enters through the bite of an infected mosquito, multiplies in local lymph nodes and is then released into the blood to be localized in the liver, spleen, kidneys and bone marrow. After an incubation period of 3-6 days, there is high fever, jaundice, albuminuria, bleeding from nose, gum, haematemesis and melena.

The high mortality rate in severe cases is due to liver and kidney failure. Yellow fever is endemic in equatorial countries of Africa and South America.

Mode of Transmission
= Cycle of

Two major epidemiologic cycles are recognized

1. **Jungle yellow fever** is primarily a disease of monkeys; the disease is transmitted from monkey to monkey by *Aedes africanus* mosquito in Africa and *Haemagogus* in South America. Man is infected accidentally during his work in forests (wood cutters, road builders) and carries the infection to urban areas.
2. **Urban yellow fever** involves man to man transmission by *Aedes aegypti* which breeds in stagnant water near houses.

Diagnosis

1. Isolation of the virus from blood during fever by intracerebral inoculation of mice.
2. Detection of the virus RNA by reverse transcriptase PCR.
3. Detection of rising antibody titre by neutralization (NT), CF or HI tests.

Prevention and control

- The use of insecticides to kill adult mosquitoes and the elimination of their breeding sites.
- Yellow fever vaccine: It is a live attenuated virus grown in chick embryo, and known as **17D Vaccine**. It is given as a single injection subcutaneously. It is very effective and protection persists for 10 years. It is given to travellers to endemic areas.

3. Dengue Fever

by Mosquito

The disease is a febrile illness with severe pain in the bones, muscles and joints (breakbone fever), headache and skin rash. Complete recovery is the rule.

The virus is a member of the *Flaviviridae* family with no cross immunity with yellow fever virus. There are four serotypes of the virus.

The incubation period is 5-6 days. Monkey is probably the main reservoir of infection and the main vector is *Aedes aegypti* mosquito.

Dengue haemorrhagic fever is a more severe syndrome affecting mainly children. The acute onset of fever is accompanied by haematemesis, melena, renal involvement and shock with case fatality rate of 50%.



4. Rift Valley Fever

The causative virus is a member of *Bunyaviridae* family and was responsible for severe epizootics in sheep and cattle in Sudan, Egypt and South Africa.

An outbreak of RVF occurred in Egypt during 1977 and resulted in 600 human deaths. Man is infected through contact with infected animals as well as by mosquito. The human disease is a febrile illness with headache, nausea, vomiting and haemorrhage.

Prevention and control

- Movements of animals should be restricted during epizootics.
- Vaccination with killed virus or attenuated live virus vaccines to prevent transmission to both humans and animals.

5. Sandfly Fever

The causative virus is distributed in countries bordering the Mediterranean sea, where the sandfly *Phlebotomus papatasi* exists. Clinical picture consists of headache, malaise, nausea, fever, photophobia, and stiffness of the neck and back. The disease is mild with complete recovery.

Roboviruses (Rodent Borne Viruses) (X)

The infectious agents are serotypes within the families of arboviruses but are not transmitted by arthropods. Rodents are the main reservoir of the virus and transmit infection to man directly.

The common clinical features are fever, petechial haemorrhage or purpura, bleeding from the gastrointestinal, genitourinary tracts and nose, shock and death.

Hantavirus genus belongs to the *Bunyaviridae* family and includes:

1. **Hantaan virus:** It causes haemorrhagic fever with renal failure. The reservoir of infection is rat. Rats on ships are responsible for worldwide spread of the virus.

2. **Hanta virus:** The reservoir of infection is rodents, which excrete the virus in urine and stools. Man is infected by inhalation of dust contaminated with these excreta. Man to man transmission does not occur.

The virus was first recognized in an outbreak in USA in 1993 as respiratory distress syndrome with high fatality rates.

Filoviridae

Marburg and Ebola viruses

These are two antigenically distinct viruses belonging to the *Filoviridae* family and appear as filaments of varying lengths. They cause African haemorrhagic fever.

(x)

- **Marburg virus** disease was recognized in 1976 among laboratory workers exposed to tissues of African green monkey. Man to man transmission occurs with high mortality rate.
- **Ebola virus** was recognized in Zaire as an epidemic of haemorrhagic fever, diarrhoea, vomiting, muscle pain and prostration. The virus is extremely virulent and hospital staff members are highly susceptible through close contact with patients, their blood or excreta.

Arenaviridae

Lassa fever virus: Infection occurs by water-contaminated by urine and saliva of infected rats which are the reservoir of infection. It was recognized in the Nigerian Village of Lassa. Man to man transmission occurs especially in hospitals and medical staff is highly exposed.

There is an acute onset of high fever, severe myalgia, haemorrhagic skin rash, pneumonia and damage of heart and kidney with 10-50% mortality rate.

Junin and Machupo viruses: They cause haemorrhagic fever in South America (Argentina and Bolivia). Infections occur among workers in maize and wheat fields who are exposed to the reservoir (rodents).

Table (10): Classification of Arboviruses and Roboviruses.

Family	Genus	Important Viruses	Characteristics of the virus
<i>Togaviridae</i>	<i>Alphavirus</i>	Western equine encephalitis (WEE), Eastern equine encephalitis (EEE), Venezuelan equine encephalitis (VEE), Sindbis virus	Enveloped (+)ssRNA
<i>Flaviviridae</i>	<i>Flavivirus</i>	Brazilian encephalitis, St. Louis encephalitis, Japanese B encephalitis, Russian spring-summer Encephalitis, Yellow fever, Dengue fever, West Nile	
<i>Bunyaviridae</i>	<i>Bunyavirus</i> <i>Phlebovirus</i> <i>Hantavirus</i>	Oropouche, Rift Valley fever (RVF) Sandfly fever Haemorrhagic fever viruses: Hantavirus, Hantaan virus	Enveloped (-) ssRNA
<i>Reoviridae</i>	<i>Coltivirus</i>	Colorado-tick fever	Non-enveloped dsRNA
<i>Arenaviridae</i>	<i>Arenavirus</i>	Haemorrhagic fever viruses: Junin, Lassa and Machupo viruses	Enveloped (-) ssRNA
<i>Filoviridae</i>	<i>Filovirus</i>	Haemorrhagic fever viruses: Marburg and Ebola viruses	

Chapter 11

DNA viruses

HERPESVIRUSES

A large family of DNA viruses, having an icosahedral protein capsid and a lipid envelope. Spikes of viral glycoproteins project from the envelope. Herpesviruses have the capacity to establish life-long latent infections from which virus may be reactivated.

Herpesviruses that infect humans

1. Herpes simplex virus (HSV).
2. Varicella-Zoster virus (VZV).
3. Epstein-Barr virus (EBV).
4. Cytomegalovirus (CMV).
5. Human herpesvirus 6 (HHV6).
6. Human herpesvirus 7 (HHV7).
7. Human herpesvirus 8 (HHV8).

HSV₁ & HSV₂ . ن. ١, ٢

Herpes Simplex Virus (HSV)

There are two distinct types of HSV, namely type 1 (HSV-1) and type 2 (HSV-2). They are similar in morphology and structure, but can be distinguished by the following:

1. Location of the lesion: Lesions caused by HSV-1 are, in general, above the waist, whereas those caused by HSV-2 are below the waist.
2. Antigenicity: HSV-1 and 2 can be differentiated by type-specific monoclonal antibodies.
3. Restriction endonuclease pattern of their genome DNA.

Pathogenesis and clinical picture

Primary infections: i.e. exposure to the virus for the first time. HSV is probably transmitted by direct contact. Primary infection usually involves the mucous membrane of the mouth. However, it may include lips, skin of face, nose, eyes and genital tract. Replication occurs at the site of the entry of the virus in the epithelium. Clinically, the disease appears as vesicles on an erythematous base. The vesicles rupture and its contents dry to form crust which heals within 7-10 days without scarring.

Latent infections: From the site of infection, virus particles are transported along the axon to the sensory (dorsal root) ganglion by retrograde axonal flow where some virus particles are able to establish a latent infection. In HSV-1 latency is in the trigeminal ganglion while in HSV-2 latency is in the sacral ganglia. Latency remains for the life time of the host.

Reactivation and recurrence: Reactivation of the latent virus may be restricted to asymptomatic shedding of the virus or may produce clinically obvious disease. Recurrent lesions will manifest at any site innervated by the affected neurons.



Reactivation of the virus is provoked by various stimuli as: fever, exposure to UV, sunlight, trauma, stress, immuno-suppression and in case of HSV-2 by sexual intercourse.

The presence of specific antibody reduces the severity -but not the recurrence- of infection.

Clinical types of HSV infection

I- HSV-1 diseases

By saliva above waist

1. **Acute Gingivostomatitis**: It is the commonest classic presentation.
2. **Recurrent herpes labialis** is the reactivation of the 1st gingivostomatitis. It is also referred to as fever blisters or cold sores.
3. **Encephalitis**: It is the most serious infection.
4. **Keratoconjunctivitis**: It may lead to corneal ulcers, scarring and blindness.
5. **Herpetic whitlow** is a pustular lesion of the finger or hand (e.g. dentists).
6. **Disseminated infections**: e.g. pneumonia as in AIDS patients.

II- HSV-2 diseases

By sexual contact below waist

1. **Herpes genitalis** is the classic presentation. It manifests as extensive bilateral painful vesicular lesions in the genital area, accompanied by fever, dysuria and inguinal lymphadenopathy.
2. **Neonatal herpes** is the most serious consequence of genital herpes and is acquired during birth.
3. **Aseptic meningitis**.

Laboratory diagnosis of HSV infections

A presumptive diagnosis is made on the basis of clinical findings, but a definitive diagnosis is important in patients with disseminated herpes, encephalitis, neonatal infection, or mucocutaneous lesions in high-risk patients who are likely to benefit from specific antiviral therapy.

1. Isolation of the virus on tissue culture.
2. Detection of HSV in vesicle fluid by electron microscopy.
3. Detection of viral DNA by PCR.
4. Detection of viral antigen by direct immunofluorescence or ELISA.
5. Serological diagnosis to detect IgM antibodies that indicates recent infection or reactivation.

Treatment

- a- Topical application of idoxuridine (IDU) is used successfully in the treatment of eye and skin infections.
- b- Acyclovir and vidarabine, which inhibit viral DNA synthesis, have better therapeutic effects especially if given early. Acyclovir (Zovirax) is available for topical, oral and I.V. use.
- c- Foscarnet acts as inhibitor of HSV DNA polymerase. It is of value in treating acyclovir-resistant HSV infections.

Prevention

id

- Immunocompromised, e.g., transplant recipients, are given acyclovir to prevent viral reactivation.



- Caesarean section is recommended for mothers with genital HSV infection to avoid neonatal infection.
- Recombinant vaccines that may help in preventing primary herpes are under investigations.

Varicella-Zoster Virus (VZV)

Infection with VZV presents in two clinical forms: The primary infection; varicella (or chickenpox) is a generalized eruption, whereas the reactivation infection; zoster (or shingles) is localized. There is only one antigenic type of VZV.

Pathogenesis and clinical features

1. **Varicella (chickenpox)** The virus is thought to enter, *by droplet infection*, through the upper respiratory tract. After an incubation period of about **2 weeks**, the typical rash of varicella appears first on the trunk then spread to the face and limbs. The skin rash is initially macular and rapidly evolves through papules to clear vesicles. The vesicles changes to pustules which dry to form scabs which heal without scar formation.

The patient is usually a child 4-10 years old. The disease usually runs a benign course. Some cases are complicated by secondary infection of skin, pneumonia, or cerebellar ataxia. Pregnant women infected in the first half of pregnancy may pass infection to the foetus which at birth may show **foetal varicella syndrome** with high mortality rate.

2. **Zoster (shingles)** It results from reactivation of latent varicella infection in the neurons. The virus reaches the ganglion from the periphery by travelling along nerve axons or by blood during viraemic stage of varicella infection during childhood.

The disease usually occurs in older people and manifests as painful vesicular eruption, unilateral and confined to one dermatome, usually thoracic or lumbar. The condition may follow trauma to the spinal cord or may complicate lymphomas, leukaemia or immunosuppression.

Treatment

Acyclovir given I.V. is effective in the treatment of varicella and zoster. It is not used for all cases, but indicated in the following conditions:

1. Immunocompromised patients.
2. Ophthalmic zoster to avoid corneal scarring.
3. Varicella complicated by pneumonia.
4. Neonatal infection.

Prevention and control

1. Acyclovir and interferon are given to immune deficient children.
2. VZ immune globulin (VZIG) of high antibody titre should be given to contacts of cases to prevent development of disease.
3. Live attenuated varicella vaccine is given as 2 doses for children 1-12 years of age. For persons aged 13 years and older, the minimum interval between doses is 28 days.

* Infectious mononucleosis
= glandular fever

Epstein-Barr Virus (EBV)

Infection with EBV is widespread and most infections are asymptomatic. The link between EBV and a variety of lymphoproliferative diseases is now clear.

1. EBV is the causative organism of the classical infectious mononucleosis (glandular fever).
2. Other EBV-associated diseases are:
 - Nasopharyngeal carcinoma.
 - Burkitt's lymphoma.
 - Oral hairy leukoplakia (seen in AIDS patients)
 - Hodgkin's disease.
 - T-cell lymphoma.

Carcinogenic Virus
* targets: B lymphocytes

The virus is excreted and transmitted via the saliva, and multiply in the oropharyngeal mucosa. EBV receptors are also present on B-lymphocytes.

Infectious Mononucleosis (Glandular Fever)

Pathogenesis and clinical features

After an incubation period of 30-50 days, the patient complains of fever, sore throat, skin rash, and cervical lymphadenopathy which becomes generalized with or without hepatosplenomegaly.

The virus attacks B cells and leads to polyclonal activation that result in the appearance of antibodies directed against a wide range of self and heterophil antigens. Infection of B lymphocytes is followed by a marked T cell response which is detected as large number of atypical lymphocytes in the peripheral blood.

Laboratory diagnosis

1. **Blood picture:** A high total leucocytic count (up to 25,000/cmm) with predominance of monocytes and atypical lymphocytes.
2. **Detection of heterophil antibodies:** which agglutinate horse or sheep red cells (Paul Bunnell test or monospot test).
3. **Definitive diagnosis** requires the demonstration of IgM to EBV capsid antigen or rising titre of IgG to both viral capsid antigen (VCA) or EB nuclear antigen (EBNA).
4. **Detection of EBV nucleic acids** in patient's saliva or throat washing using DNA probes.

Prevention and control

A subunit vaccine based on major viral glycoprotein is under trial.

81 Alk. J. J. J.
Jae oral
Bec enveloped

Cytomegalovirus (CMV)

The name "cytomegalovirus" was chosen on account of the swollen state of virus-infected cells. The virus is widespread.

Primary infections occur in 40-60% of individuals and the virus persists in the host for life (latent infection). Reactivation is common. Infection may be transplacental (congenital) or by other methods as close contact, sexual intercourse, breast feeding, blood transfusion and organ transplantation.

Clinical features: Most infections are asymptomatic:

- ① **Congenital Infection:** It may cause still birth or abortion. In *5% of infected babies, congenital abnormalities occur "cytomegalic inclusion disease". The most common features of the syndrome are growth retardation, microcephaly, hepatosplenomegaly, thrombocytopenia and blindness.
- ② **Mononucleosis syndrome;** similar to that caused by EBV. However, pharyngitis and lymphadenopathy are unusual and heterophil antibodies are not found.
- ③ **Infection in immunocompromised patients;** may manifest as pneumonitis, encephalitis, hepatitis, retinitis ... etc.

Laboratory diagnosis

1. Isolation of the virus from throat washings or urine on tissue culture.
2. Detection of viral DNA by PCR or hybridization assay.
3. Detection of viral antigens in urine or saliva.
4. Serodiagnosis: Detection of CMV IgM or rising titre of IgG by EIA or latex agglutination assay.

Treatment

Ganciclovir has been used successfully in treatment of CMV infections in immunosuppressed patients.

Prevention and control

- Screening blood donors and organ donors and exclusion of seropositive ones.
- Vaccines are under trial.

Human Herpes Virus 6 and 7 (HHV6 and HHV7)

These are recently described viruses. **HHV6** is the cause of a common disease of infancy called Exanthem subitum (roseola infantum or sixth disease), characterized by high fever and skin rash. **HHV7** is associated with persistent salivary glands infection.

Human Herpes Virus 8 (Kaposi's Sarcoma-Related Herpesvirus)

This new virus was identified in 1994 from tissue of Kaposi's sarcoma in patients with AIDS.

Chapter 12

ADENOVIRUSES

used as cloning vectors

Adenoviruses are a frequent cause of acute upper respiratory tract infections, in addition to other infections. They were isolated from adenoidal tissue of children, hence the name adenovirus. Human adenoviruses are divided into 6 groups, A to F comprising 51 serotypes.

Characteristic features

- **Widespread** in nature.
- **Inclusion bodies:** The viruses replicate in the cell nucleus forming inclusion bodies. These form the basis of latent infection.
- **Latent infection and reactivation;** can occur in lymphoid tissues. Reactivation is caused by immunosuppression, e.g. in AIDS patients.
- **Oncogenic potential:** Some strains induce tumours in newborn rodents and cell transformation of tissue culture cells. Adenovirus oncogenesis has never been observed in humans.
- **Adenovirus genome:** The adenovirus genome is easily manipulated *in vitro*. So, it can be used as a vector to carry and express foreign genes for therapeutic purposes (gene therapy) or for vaccination.

Morphology

Human adenoviruses have a simple construction and consist of a capsid, fibres, a core, and associated proteins. Virions are icosahedral **nonenveloped**, 70-90 nm in diameter with **double stranded DNA** genome. Adenoviruses are the only viruses with fibres protruding from the capsid. The fibre is the organ of attachment, a strong haemagglutinin and toxic to human cells.

Adenovirus infections in humans

Adenovirus illnesses occur most commonly among school-aged children. Approximately 50% of the infections are asymptomatic, self-limiting (within 2 weeks), and induce long-lasting type-specific immunity. Transmission occurs through respiratory droplets, faecal-oral route, and contact either by hand-to-eye or sexual. Incubation period is 5 to 8 days.

Adenovirus serotypes 1-7 and 21 are the most common types accounting for most adenovirus infections in humans.

A- Respiratory diseases: They include 5 syndromes:

1. Acute febrile pharyngitis.
2. Pharyngoconjunctival fever.
3. Acute respiratory disease occurring in epidemic form especially among military recruits. It is characterized by pharyngitis, fever, cough, and malaise.
4. Adenoviral pneumonia represents 10% of pneumonias in childhood.
5. Pertussis syndrome caused by group C.



B- Eye infections:

1. Swimming pool conjunctivitis (pink eye).
2. Follicular conjunctivitis resembling chlamydial conjunctivitis.
3. Epidemic keratoconjunctivitis is the most serious. The disease is highly infectious and may leave opacities in the cornea.
4. Acute haemorrhagic conjunctivitis.

C- Gastrointestinal disease:

1. infantile gastroenteritis.
2. Intussusception of infancy.

D- Central nervous system infections; meningitis and encephalitis.

E- Genitourinary infections:

1. Acute haemorrhagic cystitis in children.
2. Venereal disease (orchitis, cervicitis, urethritis and ulcers on external genitalia similar to herpetic lesions).

F- Other diseases:

1. Hepatitis in children with liver transplants.
2. Infections in the immunocompromised including AIDS patients (pneumonia and disseminated disease).
3. Nosocomial infections: Adenoviruses cause 10% of pneumonia cases in hospitalized children.

Laboratory diagnosis

Adenovirus infections are ideally diagnosed by isolation of the virus from clinical specimens. A fourfold or greater rise of antibody titre is a good evidence of infection.

Treatment

Antiviral agents have generally been ineffective against adenovirus infections. Intravenous ribavirin is a potential treatment.

Prevention

- Chlorination of swimming pools, drinking water, wastewater.
- High hygiene standards in ophthalmology practice.
- Measures to prevent nosocomial transmission.
- Vaccination: No vaccine available.

Chapter 13

HUMAN PARVOVIRUSES

The **Parvoviridae** family contains small, non-enveloped, ssDNA viruses with icosahedral capsids. Medically important genera are:

1. **Dependovirus** (e.g. adeno-associated virus) is a defective virus and requires helper virus for its own replication. There is no specific disease caused by dependoviruses.
2. **Erythrovirus** is capable of autonomous replication, which is independent of helper virus. **Parvovirus B19** is the only member, and is the only parvovirus that is a human pathogen. It is associated with several defined clinical syndromes. Human erythroid progenitor cells are the specific host cells, where the virus replicates inside the nucleus.

Pathogenesis of Parvovirus B19

Infection is transmitted by respiratory secretions and saliva, transplacental infection of the foetus, and blood transfusion or blood product therapy. Humans are the natural reservoir.

Most of the infections are asymptomatic. Human infections include:

1. Erythema infectiosum (slapped-cheeks syndrome, fifth disease), common.
2. Transient Aplastic Crisis (TAC), common.
3. Foetal infections leading to foetal hydrops and foetal loss.
4. Chronic B19 infections in the immunocompromised patients such as chronic anaemia, leukopenia, or thrombocytopenia.
5. Arthritis in adults resembling rheumatoid arthritis

Anaemia is due to B19 infection of the red blood cell precursors. The rash associated with erythema infectiosum is due to infection of the endothelial cells as well as deposition of immune complexes. These immune complexes also contribute to the arthritis seen in adults. Infection provides lifelong immunity.

Laboratory diagnosis

Cultivation of parvovirus B19 is difficult and not routinely used for diagnosis. Diagnosis is made either by direct detection of B19 antigen or DNA in clinical material, or by serologic tests.

Treatment and prevention

There is no specific antiviral drug. Pooled immunoglobulins may be useful in chronic B19 infections in immunocompromised patients. A recombinant safe human parvovirus B19 vaccine composed of capsid proteins is under evaluation and it is successful until now in 3 doses, at 0, 1, and 6 months. All volunteers developed neutralizing antibody with sustained immunity.

* Vaccine important before sexual life

Chapter 14

Human Papillomavirus

16, 18 = cervical cancer

6, 11 = genital warts

not cultivated

Recombinant Vaccine IM 3 doses 6m

Human papillomaviruses (HPVs) are small non-enveloped epitheliotropic dsDNA viruses. They may induce benign lesions of the skin (warts) and mucocutaneous junctions. Some HPVs are implicated in development of epithelial malignancies, especially cancer of the uterine cervix.

HPVs replicate in the nucleus of squamous epithelial cells. The genome is contained within a spherical protein capsid with 7 early proteins (E1 to E7), and 2 late structural proteins (L1 and L2). Based on L1 gene sequence difference there are more than 100 HPVs genotypes. Important types include:

- The high risk types HPV 16 & 18 associated with cervical cancer.
- HPV 6 & 11 cause most genital warts.

Transmission

Transmission of HPVs is by direct or sexual contact.

Pathogenesis

HPV produces a chronic infection of basal cell layer of the stratified squamous epithelium. HPV DNA persists in a non-integrated (episomal) form in normal infected cells but is integrated in the host cell chromosome in the high-grade dysplasia cells. Integration of HPV DNA leads to high expression of E6 and E7 genes leading to overproduction of E6 and E7 proteins leading to:

1. Inactivation of the two tumor suppressor genes p53 and retinoblastoma (pRB)
2. Induction of abnormal mitosis.

Laboratory Diagnosis

1. HPV cannot be cultivated to diagnose infections.
2. The following methods are used for cervical cancer screening. Cervical swabs or biopsy samples are subjected to:
 - a) Cytology (Pap smear)
 - b) Detection of cervical biomarkers: include E6 and E7
 - c) Hybridization or PCR molecular assays to detect HPV L1 DNA sequences, E6 or E7 mRNA

Treatment

There is no specific antiviral therapy

HPV vaccination

It is done by the recent quadrivalent HPV (types 6, 11, 16 & 18) vaccine. It protects against development of most cervical cancers and genital warts through prevention of infection by the four HPV types. It is made from recombinant noninfectious HPV-like particles (VLP). It protects by inducing serum neutralizing antibodies.

It is administered intramuscularly in a 3-dose schedule over a 6-month period. The first dose is administered to females at age 11 or 12 years, the second dose after 2 months and the third dose 6 months after the first dose. However, vaccine series can be started at 9 years of age. Catch-up vaccination is recommended for 13 through 26

year old females. It is preferable to give the vaccine before the beginning of active sexual life before contracting HPV.

Human Polyomaviruses

JC virus (JCV), BK virus (BKV), and Simian virus (SV40) are members of the family *Polyomaviridae* containing small (40 nm) virions with dsDNA genome. JCV and BKV frequently cause inapparent persistent infections, which may reactivate under immunosuppression and then result in overt disease as shown below:

Virus name	Transmission	Signs & symptoms	Important disease
BKV (<i>Polyomavirus hominis</i> 1)	<u>Respiratory route,</u> <u>contaminated food</u> <u>or water</u>	<u>Nephropathy</u>	Hemorrhagic cystitis and nephritis in; transplant patients
JCV (<i>Polyomavirus hominis</i> 2)		Latent in the lymphocytes, urogenital tract, brain	Progressive multifocal: leukoencephalopathy (PML)

Chapter 15

POXVIRUSES

Important features

1. They are brick-shaped, dsDNA viruses.
2. They can be seen by the light microscope because of their large size.
3. They contain a DNA-dependent RNA polymerase, not found in other DNA viruses because the virus replicates in the cytoplasm.

Poxviruses include:

1. Smallpox (variola or alastrim) virus.
2. Vaccinia virus and cowpox virus belong to *Orthopoxvirus* genus. Members of *Orthopoxvirus* cross-react and elicit neutralizing antibodies. Therefore, vaccinia virus was used in immunization against smallpox.
3. Molluscum contagiosum virus belongs to a different genus.

Smallpox

The last endemic case occurred in 1977 and in 1980 the WHO General Assembly certified that smallpox had been eradicated. The importance of smallpox should not be forgotten because:

1. It had a marked impact on the development of civilization.
2. It is the first disease to be controlled by immunization.
3. It is the first virus disease for which chemoprophylaxis (Methisazone) was available.
4. It is the first to be eradicated.

Prevention

The disease was eradicated by the global use of the live attenuated vaccinia virus vaccine. The success of the vaccine in **eradicating** smallpox was dependent upon five critical factors:

1. Smallpox has a single, stable serotype.
2. There is no animal reservoir, and humans are the only host.
3. The antibody response is prompt.
4. The disease is easily recognized clinically.
5. There is no carrier state or subclinical infection.

Molluscum contagiosum virus (MCV)

- It causes wart-like benign tumours of the skin or mucous membranes.
- It is different from warts caused by papillomavirus.
- MCV is transmitted by close personal contact, including sexual contact. Infection is common in children, and the lesions can be widespread in patients with reduced cell-mediated immunity.
- There is no specific antiviral treatment and no vaccine.

Chapter 16

SLOW AND UNCONVENTIONAL VIRUS DISEASES

Slow infection is a term used to describe a disease with prolonged incubation period ranging from several months to decades. Slow infections are caused by two classes of infectious agents:

1. Conventional viruses.
2. Unconventional transmissible agents (prions).

These infectious agents cause degenerative diseases of the central nervous system in humans and the clinical course usually progresses to death.

Table (11) lists some slow infections caused by conventional viruses, and table (12) lists slow infections of the unconventional agents:

Table (11): Slow infections of conventional viruses.

Disease	Virus
Subacute sclerosing panencephalitis (SSPE)	Measles virus
Progressive panencephalitis (congenital rubella)	Togavirus
Progressive multifocal leukoencephalopathy (PML)	Papova virus (JC virus)
Cytomegalovirus brain infection	Cytomegalovirus
Acquired Immunodeficiency Syndrome (AIDS)	HIV

Table (12): Slow infections of unconventional agents (Prions).

Disease	Primary Host
Creutzfeldt-Jacob disease (CJD)	Man
Kuru	Man
Gerstmann-Struassler Scheinker Syndrome (GSSS)	Man
Scrapie	Sheep
Bovine spongiform encephalopathy (mad cow)	Cattle and cows

The transmissible agent in these diseases is a protein called prion protein (PrP), devoid of any nucleic acid.

Characteristics of prion agent and diseases

- Prions are infectious pathogenic particles that lack nucleic acid, sometimes described as subviral agents. They essentially consist of a scrape-like prion protein (PrP^{Sc}), an abnormally folded, protease resistant, form of a normal cellular prion protein (PrP^{C}).
 - The infectivity of prions is due to recruitment of conformational conversion of cellular prion protein (PrP^{C}) into disease-associated PrP^{Sc} .
- Prions are extremely resistant to inactivation by sterilization processes and disinfecting agents. Inactivation could be achieved by steam sterilizers at 134°C for 30 minutes, or exposure to high concentration of sodium hypochlorite ($> 5.25\%$ fresh household bleach solution) or sodium hydroxide (1N solution) for 1 hour.



- They have long I.P., gradual onset and progressive fatal course.
- They are transmitted by inoculation or ingestion of diseased brain and other CNS tissues or eye balls. Corneal transplant and transplant of dura matter are other methods of transmission.
- Most cases occur as sporadic disorders. About 10-15% occur as inherited disorders.
- The disease is confined to the CNS with spongiform changes.
- The clinical features are progressive cerebellar syndrome with dementia in the final stages of illness.
- No evidence of host immune response, or inflammatory reactions.
- There is no antimicrobial therapy for these diseases.
- Immunotherapy with anti-PrP antibodies may represent a promising therapy.

Chapter 17

TUMOUR VIRUSES AND ONCOGENESIS

DNA
RNA

Tumour viruses are those viruses which when introduced into an appropriate host can produce tumour. It is well proved that viruses cause cancer in animals. However, proving causal relationship between viruses and human cancer is controversial and is usually based on evidences as:

1. The virus can be isolated from tumor cells at some stages of development.
2. Detection of viral nucleic acid or viral encoded protein in tumour cells.
3. The ability of the virus to cause transformation of cultured cells or tumour in laboratory animals.
4. Decrease in incidence of some tumours by prevention of the related virus.

Tumour viruses can cause cellular transformation which is known as stable heritable changes of cell characters.

Transformed cells contain either the whole or part of the viral genome usually integrated into the cell DNA. Sometimes, it is present as a free plasmid-like entity. Transformed cells do not produce viruses and detection of the integrated specific viral sequence can only be done by recombinant DNA techniques.

Mechanism of cell transformation

Tumour viruses mediate changes in cell behavior by means of a limited amount of genetic information integrated in the cell chromosome:

1. DNA viruses: A portion of viral DNA becomes integrated in the host cell genome.
2. RNA viruses (e.g., retroviruses): The reverse transcriptase makes a DNA copy of the viral RNA which is then integrated into the cell genome.

The integrated viral genes then cause transformation through:

1. The virus introduces a new "**transforming gene**" into the cell, (viral oncogene).
2. The virus induces or alters the expression of a preexisting cellular gene "**proto-oncogene**".
3. Viral proteins may inactivate tumour suppressor genes (**anti-oncogenes**). The pRB gene and the P53 gene are altered in at least 50% of human tumours.

Tumour viruses

I. DNA viruses

1. Herpes viruses
 - a- Herpes simplex 1 & 2 can cause transformation associated with cancer cervix.
 - b- Epstein Barr virus is linked to Burkitt's lymphoma.
 - c- Human Herpes virus 8 is related to Kaposi's sarcoma.
2. Hepatitis B virus is strongly implicated in development of hepatocellular carcinoma.
3. Human papilloma virus can cause warts, laryngeal papilloma or cancer cervix.
4. Adenoviruses; can cause transformation in rodent cells. No association of adenoviruses with human neoplasms has been found.
5. Poxviruses; molluscum contagiosum produces benign growths in human skin.

II. RNA viruses

1. HCV: is also strongly related to hepatocellular carcinoma + chronic hepatitis
2. Human T-cell lymphotropic virus (HTLV-1) is linked to adult T cell leukemia.

APPLIED MICROBIOLOGY

Chapter 18

NORMAL FLORA OF THE HUMAN BODY

Normal flora denotes the population of microorganisms that inhabit the skin and mucous membranes of healthy normal people. These organisms are arranged into 2 groups:

1. **Resident flora** consists of relatively fixed types of organisms regularly found in a given site at a given age. If disturbed, it promptly reestablishes itself.
2. **Transient flora** consists of non pathogenic or potentially pathogenic organisms found for some time and derived from the environment. They do not establish themselves permanently on the surface, and do not produce disease except when the resident flora is disturbed.

Role of resident flora

Resident flora is not essential for life, yet in certain areas it plays a definite role in maintaining health and normal functions, e.g.:

1. Members of resident flora in the intestinal tract synthesize vitamin K and help in the absorption of nutrients.
2. Prevent colonization by pathogens through "bacterial interference". This may be due to competition for receptors or binding sites on host cells, inhibition by toxic products or bacteriocin production or other mechanisms.

Normal flora may produce disease under certain conditions

1. Changes of their normal habitat e.g.:
 - a- Viridans streptococci are normal resident flora of the upper respiratory tract and can cause infective endocarditis in predisposed heart.
 - b- *E. coli*, normal flora of the intestine, is the most common cause of urinary tract infections.
2. Disturbance of the resident flora, e.g. by broad spectrum antibiotics, may produce superinfection.

Normal flora of the skin

It varies in different anatomical sites. It includes:

- *Staph. epidermidis*.
- Diphtheroids and propionibacterium.
- Viridans streptococci.
- Enterococci in the perineum.
- Fungi and yeasts in skin folds.
- Non-tuberculous mycobacteria.

Normal flora of mouth, throat, and pharynx

- Gram positive cocci: *Staph. epidermidis*, viridans streptococci and enterococci.
- Gram negative cocci of the commensal neisseria and moraxella.
- Diphtheroids.
- Spirochaetes: e.g. *Trep. macrodentium* and *Trep. microdentium*.
- Yeasts (candida species).
- Anaerobic bacteria, specially with bad oral hygiene and may be responsible for a foul smelling, these are:
 - Lactobacilli.
 - Prevotella specially, *P. melaninogenicus*.
 - Actinomyces.
 - Fusobacteria.

Normal flora of the intestine

Normally, the stomach is sterile due to its acidity. The duodenum and upper part of small intestines are also sterile. The lower part of small intestine and the colon contain a large amount of bacterial flora varying according to age and diet. These include:

A- **Anaerobic bacteria:** Form up to 99% of the intestinal flora, e.g.:

- *Bacteroides fragilis*.
- *Clostridium* especially *Cl. perfringens*.
- Peptostreptococci.
- Lactobacilli especially in infants.

B- **Aerobic and facultative anaerobic bacteria:** form only 1-4% of the flora, these include:

- *E.coli* and some other members of *Enterobacteriaceae*.
- *Pseudomonas*.
- Enterococci (*E. faecalis*).

C- **Yeasts**, e.g. *Candida species*.

Normal flora of the vagina

In the normal adult vagina, *Lact. doderleins* usually predominates and is responsible for the vaginal acidity which resists infections. If lactobacilli diminish in number, e.g. after menopause or antibiotic treatment, mixed flora predominates which includes:

- *Staph. epidermidis*.
- Group B haemolytic streptococci.
- Peptostreptococci.
- *Gardnerella vaginalis*.
- *Ureaplasma urealyticum*
- Yeasts (*Candida albicans*).

Carriers

A carrier is an apparently healthy individual harbouring a pathogenic organism, without having clinical manifestations, and can transmit this organism to others.

Carriers are more dangerous than cases as a source of infection. This is because:

1. They move freely among people without being detected.
2. They carry the organism in the interepidemic periods.

According to the duration of the carriage state, carriers may be: (a) transient carriers e.g. during the incubation period and early convalescence, or (b) chronic carriers e.g. hepatitis-B virus. The organism may be discharged from the carrier in an intermittent or a continuous manner.

The disease conditions in which carriers play an important role include:

1. Enteric fever (gall bladder).
2. Cholera (intestine).
3. Epidemic cerebrospinal meningitis (nasopharynx).
4. Diphtheria (throat).
5. Hepatitis B virus infection (blood).
6. *Staph. aureus* infections (skin and nose).

Chapter 19

ANAEROBIC INFECTIONS

Classification of anaerobes

- I. Spore forming anaerobes: these include the clostridium group.
- II. Non-spore-forming anaerobes (cocci and bacilli): (Table 13). Infections caused by this group are more common than infections caused by spore-forming clostridia.
- III. Spirochaetes: *Treponema*.

Table (13): Important nonspore-forming anaerobic bacteria.

Gram-positive bacteria		Gram-negative bacteria	
Cocci	Bacilli	Cocci	Bacilli
- Peptococci - Peptostreptococci	- <i>Actinomyces</i> - <i>Propionibacterium</i> - Lactobacilli	- <i>Veillonella</i>	- <i>Bacteroides</i> - <i>Fusobacterium</i>

Predisposing factors for anaerobic infections

Anaerobic bacteria will not multiply in tissue to cause infection unless factors are present which will lower the oxygen tension or which are associated with a lowering of the oxidation-reduction potential (Eh) through the production of reducing substances. These predisposing factors include:

1. Trauma: Dead tissues specially in deep wounds as car accidents.
2. Impaired blood supply: e.g. ischemic arterial disease of foot or leg of a diabetic patient.
3. Presence of other organisms: e.g. *E. coli* or *Staph. aureus* which will consume O₂, thus lowering the Eh. of the area.
Anaerobic infections are often mixed, e.g. aerobes and anaerobes or more than one species of anaerobes together.
4. Foreign bodies: e.g. soil as in car accident or war wounds.

Features of anaerobic infections

1. Production of a large amount of foul-smelling pus.
2. Proximity of lesions to mucosal surface or portal of entry.
3. Failure to isolate organisms from pus by the usual aerobic methods (sterile pus).
4. Infection associated with necrotic tissue, deep seated abscesses or closed space, e.g. brain abscess.
5. Gas formation in the surrounding tissues detected as crepitations.
6. Failure to respond to conventional antimicrobial therapy.
7. Presence of special character, e.g. sulphur granules seen by the naked eye in the pus in case of actinomycosis or red fluorescence under U.V. light in case of prevotella infections.

Common types of anaerobic infections

1. **Respiratory tract infections:** Periodontal infections, sinusitis, mastoiditis, vincent angina, actinomycosis, lung abscess, pneumonia and empyema.
2. **Central nervous system infections:** Brain abscess, rarely meningitis.
3. **Intra-abdominal and pelvic infections:** Cholecystitis, appendicitis, liver abscess, peritonitis, pelvic actinomycosis associated with intrauterine device (IUD), septic abortion.
4. **Wounds and soft tissue infections:** Gas gangrene, necrotizing fascitis and cellulites.
5. **Bacteraemia and endocarditis:** About 5-10% of these infections are caused by anaerobes originating in the gut or the female genital tract. This may lead to septic abortion.

Laboratory diagnosis

When anaerobic infection is suspected, it is important that adequate clinical specimens are collected and transported, as soon as possible, to the laboratory, preferably before antibiotic therapy. Best samples are taken by disposable closed syringe or on swab with reduced transport media. The samples should be taken, as deep as possible, away from atmospheric oxygen and as much sample as possible.

In the laboratory, the following steps are done:

1. Macroscopic examination:
 - Sulphur granules in actinomycosis.
 - U.V. light may show brick-red fluorescence suggesting *Prevotella melaninogenica* infection.
2. Microscopic examination:

Gram stained smear may help in diagnosis of vincent angina. The absence of organism by direct microscopy does not exclude the presence of anaerobes in the provided sample.
3. Direct gas liquid chromatography (GLC): It is rapid reliable method for detection of anaerobes.
4. Culture; on blood agar and selective media freshly prepared in the same day. Incubation is done at 37°C in anaerobic jar (Gaspak system) with 10% CO₂. All anaerobic cultures are incubated for at least 48 hours, better for up to 5 days, because anaerobes are usually slow growers.

Every sample is cultured on two plates of blood agar, one incubated aerobically and the second plate incubated anaerobically. After incubation, comparison of types of colonies developed in both plates will show that anaerobic organisms will grow **only** on the plate incubated under anaerobic conditions.

Enrichment cultures: Robertson's cooked meat broth and thioglycollate media are sometimes used but are less significant than direct culture on blood agar plates.

Colonies are identified as follows:

1. Colony morphology, e.g. pigment production.
2. Morphology of the organism by Gram stain.
3. Biochemical reactions using API A (Analytical Profile Index for Anaerobes).
4. GLC to detect short chain fatty acids.
5. Serological identification, e.g. direct IF.
6. Nucleic acid probes.

Treatment of anaerobic infections

1. **Surgical treatment:** drainage of pus from abscesses, debridement, and removal of necrotic tissues may be sufficient.
2. **Antimicrobial therapy:**
 - The two most active antibiotics against all anaerobes are metronidazole and clindamycin.
 - Penicillins are active against the majority of anaerobes that cause infection above the diaphragm, such as prevotella, leptotrichia, fusiforms, anaerobic cocci, and actinomyces, but are not active against the majority of anaerobes associated with infection below the diaphragm, especially *Bacteroides fragilis*, due to the production of B-lactamase.
 - Chloramphenicol is especially effective in treatment of brain abscess as it can pass blood brain barrier.
 - Lincomycin is highly effective.
3. The treatment of the aerobic organisms accompanying anaerobes, in a mixed infection, is also necessary.

Chapter 20

INFECTION CONTROL FOR MEDICAL STUDENTS

Healthcare-associated infections (HAIs) are a serious problem in the hospitals. Therefore each hospital should have an Infection Control (IC) program designed to prevent acquisition of infections. HAI is an infection that develops, usually after 48 h, in a patient and is related to receiving healthcare.

IC program

It is an IC plan that should be designed by specialized IC persons. The plan should give a continuously updated guide to identify, assess, and reduce different risks of infections (HAIs) among patients, employees, students, and visitors in the hospitals.

The IC program should focus on the followings:

I. Protecting patients vulnerable to infection such as:

1. Patients for surgery
2. Neonates
3. Burn patients.
4. Patients on mechanical ventilator are at risk of pneumonia
5. Patient with inserted urinary catheter or vascular catheter
6. Patient on haemodialysis at risk of HCV ,HBV, HIV Infections
7. Patient with neutropenia, organ transplant patients, leukemia...
8. Patient for dental procedures
9. Patient for endoscopy

II. Preventing spread of epidemiologically significant organisms, examples:

1. Multi-drug resistant bacteria (e.g. MRSA, VRSA)
2. *Mycobacterium tuberculosis*
3. Standard bloodborne pathogens, HCV ,HBV, HIV
4. *Neisseria meningitides*
5. Avian Influenza virus A/H₅N₁

III. Hospital units / departments with high infection risks to patients:

1. Intensive care unit (ICU)
2. Neonatal Intensive care unit (NICU)
3. Burns unit
4. Delivery Room (DR)
5. Pediatric Intensive Care unit (PICU)
6. Dialysis unit
7. Emergency room (ER)
8. Dental clinic
9. Operation room & Anesthesia
10. Central Sterilization Supply Department (CSSD)
11. Preparation of nutrient formulas for parenteral nutrition of pediatric and adult patients (total parenteral nutrition, TPN).

Recommended Infection Control Practices to Prevent Transmission of Infections

1. **Hand hygiene** should be practiced by all HCWs since it is the most effective way to prevent co-transmission of HAIs.
2. **Isolation Precautions, include:**
 - a) **Standard Precautions** must be applied all the time of care irrespective of the infectious status of the patient
 - b) **Transmission-based Precautions** must be applied in addition to the Standard Precautions when a patient has a diagnosed infection:
 1. **Airborne Precautions** in patients infected with agents transmitted by airborne: pulmonary TB, chicken pox and measles
 2. **Droplet Precautions** in patients infected with agents transmitted by droplets: meningococcal meningitis, influenza, mumps, rubella...
 3. **Contact Precautions** in patients infected with agents transmitted by contact: MRSA, diarrhea, hepatitis A...
3. Education and training of HCWs, patients, family, and visitors, regarding hand hygiene and isolation precautions
4. Use surveillance data to monitor performance of IC practices
5. Use surveillance cultures when indicated
6. Management of infection outbreaks and epidemics
7. Preparation of IC **policies** based on sound evidences

HAND HYGIENE

Importance of hand hygiene

1. Many infections are transmitted on the hands of healthcare personnel.
2. It can reduce the transmission of infections – to your patients and to you.

When is hand hygiene done?

1. Immediately **before** touching a patient
2. Immediately **after** touching a patient
3. Immediately **before** aseptic technique e.g. handling a medication
4. **After** touching patient surroundings
5. Immediately **after** touching blood, body fluids or items contaminated with them
6. **After** removing used gloves on a patient

Types of hand hygiene

1. Alcohol hand rub by alcohol gel or solution: it is highly effective in killing microbes & does not irritate the skin. It should be used routinely unless hands are soiled
2. Hand wash by soap & water: is best used for soiled hands because alcohol does not act on microbes in presence of dirt
3. Surgical hand scrub when performing surgery

When gloves should be used?

1. Before touching nonintact skin, mucosa, blood or body fluids of patients
2. Touch of equipment/devices contaminated with substances noted above

3. Remove immediately after use & before leaving point of care in order not to contaminate other places

Standard Precautions

Definition: The Standard Precautions are a group of IC practices which when implemented by HCWs should reduce risk of transmission of blood borne pathogens (HBV, HCV and HIV) as well as other microorganisms among HCWs and patients.

Principle: The Standard Precautions assume that every patient is potentially infected with an organism that could be transmitted. Thus precautions should be automatically applied to all patients all the time regardless of their infection status.

Standard Precautions practices include:

- 1) **Hand hygiene** (see above)
- 2) **Personal protective equipment (PPE):** use of gloves, gown, mask ..etc.
- 3) **Cleaning, disinfection and sterilization of patient care equipment, instruments and devices.**
- 4) **Respiratory Hygiene & Cough Etiquette:** to contain respiratory secretions and prevent transmission of respiratory pathogens. persons suffering acute respiratory illness e.g., flu, are instructed to:
 - cover mouth & nose when sneezing/coughing with tissues or mask;
 - wash hands or rub with alcohol
- 5) **Care of the environment:**
 - Clean and maintain routinely environmental and clinical surfaces
 - Use of disinfectants
- 6) **Safe injection practices**
- 7) **Workers' safety and employee health** (see below)
- 8) **Proper handling of soiled linen**
- 9) **Safe disposal of infectious waste:**
 - Solid waste e.g., used gloves, pathological tissue & placenta
 - Blood and body fluids
 - Used needles and syringes

Airborne Precautions

Indications: Patients with Pulmonary TB, chickenpox, and measles

Measures:

1. Patient should be isolated in negative pressure room: so that microorganisms carried by air from the patient will not spread to other hospital areas.
2. Caring staff must use N95 mask before entering the room.

Contact Precautions

Indications: patients infected with MRSA or other multidrug resistant organisms (MDROs), or diarrhea.

Measures:

1. Patient placed in single room
2. Caring staff must use gown and gloves upon room entry.

Droplet Precautions

Indications: patients with meningitis (meningococcal), mumps, rubella, influenza.

Measures:

1. Patient placed in single room
2. Caring staff must use surgical mask upon room entry.

Employee Health

1. Occupational exposures of the healthcare workers (HCWs) to infections should be prevented by following IC guidelines.
2. When accidental exposures occur the exposed person should receive **Post-exposure prophylaxis** especially for serious infections HBV, HCV and HIV.
3. Hepatitis B vaccine and seasonal influenza vaccine should be given to HCWs

HCWs should follow the best practice to avoid exposure to patient's blood:

- ☐ Using gloves, masks, eye goggles, gown
- ☐ Avoiding unnecessary use of needles and other sharps
- ☐ Not recapping needles
- ☐ Discarding needles and sharps in puncture resistant container
- ☐ Not holding patient tissue with fingers when suturing or cutting
- ☐ Prompt cleaning blood spills by using household bleach (1000 ppm available chlorine)

What to do if you are exposed?

1. Immediately and vigorously wash the site of needle sticks with soap & water.
2. Immediately flush with water if splashes to the nose, mouth, or skin.
3. If splashes to the eyes, irrigate with clean water, saline or sterile irrigates.

What care provided to the exposed person?

1. Identification of the source person if possible.
2. Baseline lab testing done at once, then follow-up testing for 6 months
3. Checking for risk of HBV, HCV, and HIV infection.
4. Post-exposure prophylaxis (PEP) on time, if indicated.

Post-exposure prophylaxis (PEP)

A. Person exposed to HBsAg positive source: give PEP according to vaccination status

a) Unvaccinated

1. Initiate Hepatitis B vaccine series
2. Give a single dose of Hepatitis B immune globulin (HBIG) within 24 hours

b) **Incomplete vaccination:** Give one dose of HBIG immediately and complete vaccination.

c) **Vaccinated, Responder:** No treatment

d) **Vaccinated, Nonresponder:** Give two doses of HBIG, one given within 24 hours and the second, one month later.

B. Person exposed to HIV positive source: give anti-HIV drugs according to risk of infection

a) **Recommend basic 2-drug** to persons exposed to HIV patient without AIDS symptoms if minimal percutaneous injuries or mucosal exposure to small volume of blood occurred.

b) **Recommend expanded 3-drug to cases of high risk of exposures:**

1. HIV patient without AIDS symptoms if severe skin injury or mucosal exposure to large volume of blood. Drugs should be given within 2h for 28 days if tolerated by the exposed.
2. AIDS patient with any degree of percutaneous injuries or mucosal exposure

Microbiologic surveillance related to infection control in hospitals

1. Routine monitoring

- i. Spore test for monitoring the sterilization processes.
- ii. Cultures of haemodialysis water.

2. **Outbreak investigation:** Examples include outbreaks of surgical site infections and MRSA outbreaks in ICU. Except on these occasions microbiologic sampling and cultures from patients and HCWs or hospital environment are useless and recommended to be stopped. Typing of strains and tracing of the source can be obtained by colony morphology, biotype profiles, phage typing, plasmid analysis, ribotyping, chromosomal analysis and PCR.

Chapter 21

IMPORTANT CLINICAL INFECTIOUS DISEASES

Meningitis

Meningitis is the infection and inflammation of the membranes surrounding the brain and spinal cord. There are two major forms of meningitis, septic and aseptic. **Septic meningitis** is typically caused by bacteria. **Aseptic meningitis** can be caused by viruses, fungi, *Mycobacterium tuberculosis* or spirochaetes. Knowing whether a virus or a bacterium causes meningitis is important because the severity of illness and the treatment differ. Viral meningitis is generally less severe and resolves without specific treatment. Bacterial meningitis can be severe and may result in brain damage, hearing loss, or learning disability. Thus bacterial meningitis is a medical emergency that indicates prompt initiation of antibiotic treatment. For bacterial meningitis, it is also important to know which type of bacteria is causing the meningitis because antibiotics can prevent some types from spreading and infecting other people.

Bacterial Meningitis

Bacterial meningitis is most common in the very young, the very old and the immunocompromised.

Causes

A) Common causes

1. *Neisseria meningitidis* (meningococcal meningitis) is the commonest cause worldwide.
2. *Haemophilus influenzae* type b (Hib).
3. *Streptococcus pneumoniae* (pneumococcal meningitis).

B) *Mycobacterium tuberculosis* (tuberculous meningitis).

C) Less common causes

1. *Listeria monocytogenes*.
2. *Staphylococcus* species.
3. *Streptococcus* species.
4. Gram-negative enteric bacilli.
5. *Pseudomonas aeruginosa*.
6. Spirochetes: *Leptospira interrogans* (Weil's disease), *Treponema pallidum* (syphilitic meningitis in secondary syphilis and neurosyphilis in tertiary syphilis) and *Borrelia burgdorferi* (Lyme disease).

D) Neonatal meningitis

1. *Streptococcus agalactiae*.
2. *Escherichia coli* (usually having K1 antigen).
3. *Listeria monocytogenes*.
4. Gram-negative enteric bacilli including *K. pneumoniae* and *Citrobacter* spp.

Viral Meningitis

Causes

1. **Enteroviruses:** poliovirus (rare due to immunization), coxsackieviruses A, coxsackieviruses B and enterovirus type 71.
2. Herpes simplex virus type 2 (HSV-2).
3. Adenoviruses.

Fungal Meningitis

Causes

1. *Cryptococcus neoformans*.
2. *Coccidioides immitis*.

Laboratory diagnosis of meningitis

A- Specimens: CSF should be collected, by lumbar puncture, before initiation of antibiotic therapy and under strict aseptic precautions. Blood specimens for blood culture are also valuable.

B- Physical and chemical analysis of CSF: Examination of uncentrifuged CSF allows differentiation between septic and aseptic meningitis as shown in table (15)

Table (14): The CSF findings in septic and aseptic meningitis

Diagnosis	Aspect	Leucocytes per ml	Predominant leucocyte	Glucose mg/dL	Protein mg/dL
Normal	Clear	0-5	Lymphocytes	45-85	15-45
Septic (purulent or pyogenic) meningitis	Cloudy	200-20,000	Neutrophils	Low	High
Aseptic meningitis, viral	Clear	100-1000	Lymphocytes	Normal or low	Moderately high

N.B.

1. CSF glucose level must be considered in relation to blood glucose level.
2. Serum CRP can differentiate bacterial from viral meningitis. It is positive in bacterial and negative in viral meningitis.
3. In case of tuberculous meningitis, a fibrin web may develop on standing.

C- Direct detection

1. **Gram-stained smears** prepared from sediment of centrifuged CSF. Smears show Gram-negative diplococci, intracellular, in some polymorphonuclear leucocytes with *N. meningitidis*, Gram-negative coccobacilli with Hib and Gram-positive diplococci with *S. pneumoniae*.
2. Ziehl-Neelsen stain to detect AFB.

3. Examination of a fresh unstained smear by dark ground microscopy may reveal motile leptospira.
4. India ink stain in suspected cryptococcosis.
5. Direct antigen detection of *N. meningitidis*, Hib and *S. pneumoniae* by latex agglutination or fluorescent antibody test in CSF or blood.
The result is essential to direct the antimicrobial therapy and must be reported within one hour to the treating physician.
6. Molecular techniques: PCR on CSF or blood allows the rapid diagnosis of some viral agents

D- Cultivation

1. CSF deposit is plated onto chocolate and blood agar. Cultures should be incubated in a humid atmosphere with 5-10% CO₂ at 37°C.
2. Blood samples should be cultivated by the blood culture technique. Subcultures are plated on chocolate and blood agar and incubated as above. Blood culture is usually positive as bacterial meningitis is often associated with bacteraemia.
3. CSF deposit should also be cultured on Lowenstein-Jensen medium in tuberculous meningitis, on Fletcher's medium in leptospiral meningitis and on Sabouraud's agar if fungal meningitis is suspected.
4. Inoculation of CSF onto cell cultures: for isolation of the causative viral agents in suspected viral meningitis

E- Identification of cultures is carried out in a systematic way according to suspected organism.

F- Serology: It is useless for early diagnosis of bacterial meningitis. However, serological diagnosis of viral causes can be achieved by demonstrating IgM antibody or fourfold increase of IgG in paired sera.

Respiratory Tract Infections

The nose is the main portal of entry for microorganisms to the respiratory system.

I. Diseases of the upper respiratory tract

Pharyngitis, Tonsillitis and Sore Throat

Pharyngitis is seen most frequently in children from 2 years of age through adolescence.

Bacterial infections

- *Streptococcus pyogenes* (the most common bacterial cause).
- Vincent's angina.
- *Mycoplasma pneumoniae*.
- *Corynebacterium diphtheriae* (very rare due to vaccination).

Fungal infections

Candidiasis (Oral thrush).

Viral infections are the most common causes:

- Adenovirus (the most common viral cause).
- Rhinoviruses.
- Influenza viruses.
- Coronaviruses.
- Parainfluenza viruses.
- Enterovirus groups: Coxsackieviruses A (herpangina).
- Herpesviruses (rare): varicella zoster virus (VZV), Epstein Barr virus (EBV), cytomegalovirus (CMV) and herpes simplex virus (HSV-1).

Acute Otitis Media

It is infection of the middle ear. Most common causes are:

- *S. pneumoniae*.
- *H. influenzae*.
- *Moraxella catarrhalis*.
- *S. pyogenes*.
- *S. aureus*.
- Viral causes include measles virus, parainfluenza virus, and respiratory syncytial virus (RSV).

These organisms along with anaerobic bacteria and fungi are the most important pathogens in **sinusitis**.

External otitis is a common problem in swimmers especially in warm-weather months. *S. aureus* and *P. aeruginosa* are the most common agents of this condition.

II. Diseases of the lower respiratory tract (LRI)

They include tracheitis, bronchitis, bronchiolitis, pneumonitis (inflammation of the alveoli), pneumonia (when alveoli fill with pus and fluid) and pleurisy.

Patients with typical pneumonia usually complain of fever, pleuritic chest pain and productive cough with sputum but in atypical pneumonia sputum is scanty or absent. However, classification into typical or atypical is not much helpful in diagnosis since sputum may be mucoid and profuse with atypical pneumonia. Classification into community-acquired and nosocomial pneumonias is more helpful for the management of the patients.

Community-acquired pneumonia

Bacterial pneumonia

- *Streptococcus pneumoniae* is the most common bacterial cause.
- *H. influenzae*.
- *M. catarrhalis*.
- ***Mycobacterium tuberculosis***.
- *Mycoplasma pneumoniae* in school-age students through young adulthood.
- *Coxiella burnetti* : Q fever.
- **Uncommon:** *K. pneumoniae*, *P. aeruginosa*, *Legionella*, *Chlamydophila pneumoniae*, *Chlamydophila psittaci*, *S. aureus*, *S. agalactiae*.

Viral pneumonia

- Influenza A virus.
 - Respiratory syncytial virus in infants and young children.
- Viral infections may set the stage for secondary bacterial infections.

Fungal pneumonia

- *Pneumocystis jirovecii* causes disease among immunocompromised, especially AIDS patients.
- Dimorphic fungi: *Histoplasma capsulatum*, *Blastomyces dermatidis* and *Coccidioides immitis*.
- Filamentous fungi (moulds): *Aspergillus*, *Rhizopus* and *Mucor*.

Healthcare-associated pneumonia: Either early onset or late onset

A) Early onset pneumonia occurs during the first 4 days of hospitalization. It is rare and is often caused by:

1. *Moraxella catarrhalis*.
2. *H. Influenzae*.
3. *S. pneumoniae*.
4. Viruses (e.g. influenza A and B viruses or respiratory syncytial virus).

B) Late onset pneumonia is the commonest form and is caused by:

1. *Enterobacteriaceae* (40%): *K. pneumoniae*, *Serratia marcescens*...etc.
2. *Staphylococcus aureus* (25%) including methicillin-resistant *S. aureus*.
3. Gram-negative bacilli: *P. aeruginosa* (15%) and *Acinetobacter* species.
4. Filamentous fungi (moulds): *Aspergillus*.
5. Yeasts such as *Cryptococcus neoformans*.

Laboratory diagnosis

Sputum and blood specimens must be collected. Sputum can be obtained by deep cough, induction, aspiration, or bronchoalveolar lavage (BAL). Sometimes lung biopsy is required for diagnosis.

Sputum smears must be first examined by Gram stain to determine that it is a true sputum and not saliva. Purulent sputum is defined as secretions from the lungs, bronchi, or trachea that contain ≥ 25 WBCs and ≤ 10 squamous epithelial cells per low power field. Acceptable specimens are then subjected to the different methods of diagnosis to determine the aetiology of pneumonia. Positive blood cultures are very helpful in diagnosis because 30% of cases of bacterial pneumonia have bacteraemia.

Conjunctivitis

I. Bacterial causes

- *S. pneumoniae*.
- *Haemophilus aegyptius*.
- *Moraxella lacunata*.
- *Chlamydia trachomatis*.
- *Staph. aureus* causes sticky eye in neonates.
- *N. gonorrhoea* causes ophthalmia neonatorum.
- *Pseudomonas aeruginosa*: following trauma to the eye or presence of foreign bodies or operations.

II. Viral causes

- Adenoviruses.
- Herpes simplex.
- Enterovirus groups: Coxsackieviruses A, and Enterovirus type 70.

III. Fungal causes

- *Candida* and *Aspergillus* species are rare causes of corneal ulcers with serious complications.

Microbial Diseases of the Gastrointestinal Tract

I. Bacterial Diseases

- A) **Chronic gastritis and peptic ulcer** caused by *Helicobacter pylori*.
- B) **Noninvasive bacterial gastroenteritis** (noninflammatory diarrhoea): It is acute watery diarrhoea without fever. It involves the small intestine and is due to enterotoxin production. There is no mucosal destruction. It is caused by:
- *Clostridium perfringens* food poisoning.
 - *Enterotoxigenic Escherichia coli*.
 - *Bacillus cereus*: diarrhoeal form of food poisoning.
 - *Clostridium difficile*: antibiotic associated diarrhoea.
 - *Vibrio cholerae* serogroups O1 and O139: cholera.
- C) **Invasive bacterial gastroenteritis** (inflammatory diarrhoea): It is characterized by mucosal invasion with resulting inflammation due to invasive or cytotoxigenic pathogens. The illness usually involves the large intestine and may be associated with fever, abdominal pain and dysentery. Stools may be bloody and contain many leukocytes. It is caused by:
- *Salmonella* food poisoning caused by nontyphoidal *Salmonella* strains, commonly *Salmonella* Enteritidis and *Salmonella* Typhimurium.
 - *Shigella* species (bacillary dysentery).
 - *Campylobacter* and *Arcobacter* species.
 - Enteroinvasive *E. coli*.
 - Enterohemorrhagic *E. coli* (*E. coli* O157:H7).
 - *Listeria monocytogenes* food poisoning.
 - *Vibrio parahaemolyticus* food poisoning.
 - *Yersinia enterocolitica*.
 - *Aeromonas* spp.
- D) **Ingestion of preformed bacterial toxins** presenting with gastrointestinal tract symptoms with vomiting as primary symptom; diarrhoea may also be present:
- *Staphylococcus aureus* food poisoning.
 - *Bacillus cereus* food poisoning (emetic form).
- E) **Ingestion of preformed bacterial toxins** presenting with neurological manifestations:
- Botulism food poisoning (*Clostridium botulinum* toxin).
- F) **Intestinal infection presenting with systemic illness:**
- *Salmonella* Typhi and Paratyphi A, B and C (enteric fever).
 - *Campylobacter jejuni* (Guillain Barré syndrome).
 - *Listeria monocytogenes*.
 - *Vibrio vulnificus*.

II. Viral Diseases

A) Viral gastroenteritis

1. Rotavirus is the leading cause of infantile gastroenteritis.
2. Norwalk virus is the leading cause of epidemic viral gastroenteritis in school children and adults.
3. Astroviruses cause sporadic gastroenteritis in infants, children, elderly and the immunocompromised patients.
4. Adenovirus (Enteric adenoviruses serotypes 40, 41 and 42): Adenoviruses 40 and 41 represent the second most common cause of viral gastrointestinal disease in children.

B) Viral hepatitis (see below).

C) Viral infection of salivary glands and pancreas by:

1. Mumps virus.
2. Human herpes virus 7 (HHV7).

III. Fungal Diseases

1. Ergot poisoning.
2. Mushroom poisoning.
3. Aflatoxin poisoning (*Aspergillus flavus* mycotoxin).
4. Candidiasis.

Food Borne Illnesses & Food Poisoning

Patients with foodborne illnesses typically present with gastrointestinal tract symptoms (vomiting, diarrhoea, abdominal pain). However, other presentations may occur which help recognizing the aetiological agents.

Food borne illness may occur in the form of an outbreak which is defined as an incident in which two or more individuals experience a similar illness resulting from the ingestion of the same food. This definition also applies to the term food poisoning. Table (15) provides information that could be used as a guideline to identify the aetiological agents of food poisoning.

Table (15): Comparison of common types of food poisoning

Aetiology	Associated foods	Incubation period	Signs and symptoms	Duration of illness	Laboratory testing
<i>Staphylococcus aureus</i>: - (the most common) - Preformed enterotoxin	- Carbohydrate rich food e.g. cake, pasta, and koskosi. - Protein rich food like milk and its products (e.g. ice cream)	very short , 1- 6 hrs	Severe vomiting and diarrhoea, but no fever	24-48 hrs	Stools, vomitus, and food remnants can be tested for enterotoxin and cultured for <i>S. aureus</i>
<i>Salmonella</i>: <i>S. Enteritidis</i> and <i>S. Typhimurium</i> invade and replicate in the epithelial cells of intestines. No toxin	- Raw and undercooked eggs - Contaminated poultry	8-48 hrs	Severe diarrhoea, fever and abdominal cramps	4 days or more	Stools culture
<i>Clostridium perfringens</i>: multiplication and release of enterotoxin in gut	Cooked meat stored at the inappropriate temperature	8-24 hrs	Diarrhoea and abdominal cramps	~24 hrs	- Quantitative culture from the stools. - Demonstration of enterotoxin in the stools
<i>Clostridium botulinum</i>: preformed neurotoxin	Canned food, sausage, and salted fish.	12-72 hrs	Diplopia, blurred vision, and bulbar weakness; paralysis,	Variable (days to months)	Detection of botulinum toxin in serum, stools, gastric contents, or implicated food
<i>Bacillus cereus</i> a. Emetic form: a preformed heat-stable enterotoxin	Improperly refrigerated cooked or fried rice	(short incubation) 1-6 hrs	Vomiting and abdominal cramps	24 hrs	Stools and food specimens for culture and toxin identification
b. Diarrhoeal form: a heat-labile enterotoxin leading to increased cAMP	Meat	(long incubation) 6-24 hrs	Diarrhoea and abdominal cramps	24-48 hours	
<i>Listeria monocytogenes</i>: multiplication and invasion of intestinal epithelium	- Fresh soft cheese - Ready-to-eat meat	9-48 hrs for gastrointestinal symptoms	Diarrhoea, abdominal cramps, fever	Variable	Detection of antibody to listerolysin O.
<i>Vibrio parahaemolyticus</i>: Thermostable direct haemolysin	Undercooked or raw seafood, such as fish, shellfish.	2-48 hrs	Watery diarrhoea, abdominal cramps, vomiting.	2-5 days	Isolation of <i>V. parahaemolyticus</i> from stools or food

Hepatitis

The causes of hepatitis can be classified as:

(I) Microbial causes

A) Viral hepatitis

1. Viruses infecting the liver as a primary target via oral route include hepatitis A and E viruses.
2. Viruses infecting the liver as a primary target via parenteral route include hepatitis B, C, D and GBV-C viruses.
3. Viruses infecting the liver as a secondary target via parenteral route include yellow fever virus, Epstein-Barr virus and Cytomegalovirus.

B) Bacterial infections: The most common causes:

1. Q fever (*Coxiella burnetii*).
2. Leptospirosis (*Leptospira interrogans*).

C) Fungal diseases

1. Ergot poisoning.
2. Aflatoxin poisoning: *Aspergillus flavus* mycotoxin.

D) Parasitic diseases

(II) Other causes

1. **Autoimmune hepatitis:** Autoimmune hepatitis occurs when autoantibodies attack liver cells. A genetic factor may predispose some people to autoimmune hepatitis. About 70 percent of those with autoimmune hepatitis are women, most between the ages of 15 and 40. The disease is usually quite serious and, if not treated, can lead to liver cirrhosis and failure.
2. Systemic lupus erythematosus.
3. Drugs.
4. Toxins.

Urinary Tract Infections

Urinary tract infections (UTI) are common and cause significant morbidity and mortality.

Bladder urine is normally sterile.

Urinary tract infection is the presence of microorganisms in the urinary tract. Most patients with UTI have "**significant bacteriuria**", (i.e. a bacterial count greater than 10^5 bacteria per ml) in a suitably collected and well transported mid-stream sample of urine. However, occasionally in symptomatic patients, a count of 10^4 bacteria per ml, in absence of antibiotics, may be significant.

Pathogenesis

Organisms from the faecal flora usually enter the urinary tract by ascending from the perineum and peri-urethral sites (ascending infection). Rarely, the kidney can be infected by the haematogenous route, particularly after a *Staph. aureus* bacteraemia.

Infection is predisposed to by:

1. Short female urethra.
2. Sexual intercourse.
3. Senile prostatic hypertrophy.
4. Structural and neurological abnormalities of the urinary tract which are associated with residual urine, i.e. incomplete emptying of the bladder after micturation.
5. Instrumentation, e.g. urinary catheters which are the most important predisposing cause of UTI in hospital patient. It usually results in infection by antibiotic resistant bacterial strains.
6. Other host factors as diabetes mellitus and immunosuppression.

Causative organisms:

- *Escherichia coli* is the commonest cause. It accounts for up to 80% of cases in general practice and about 50% of cases in hospital practice.
- *Enterococcus faecalis*.
- *Staphylococcus saprophyticus* (particularly in young sexually active women). *not sexually transmit*
- *Proteus mirabilis*, *Klebsiella pneumoniae* and other *Enterobacteriaceae*.
- *Pseudomonas aeruginosa*.
- *Staphylococcus aureus*.
- Coagulase negative staphylococci other than *S. saprophyticus*.
- *Mycobacterium tuberculosis*.
- Adenoviruses (acute haemorrhagic cystitis in children).
- *Candida albicans*.

Laboratory diagnosis

I. Sample collection:

- After adequate cleaning of the external genitalia with tap water, and drying, a mid-stream specimen of urine is obtained in a sterile container.
- In infants, adhesive sterile bags may be used.
- Suprapubic aspiration is occasionally necessary in infants and in pregnancy.
- Catheter sample should be avoided unless the patient is already catheterized.

II. Transport to the laboratory: The urine should reach the microbiology laboratory within 2 hours of collection. If not possible, the sample should be kept at 4°C to avoid multiplication of possible contaminants which results in a false high bacterial count.

III. Microscopic examination: for the presence of pus cells, RBCs, bilharzial ova, casts, crystals, ... etc.

IV. Viable bacterial count: A measured volume of uncentrifuged urine is spread on the surface of a solid medium and incubated. Colonies are counted.

V. Culture: Urine deposit is cultured on nutrient agar, blood agar and MacConkey's medium. After incubation at 37°C for 24 hours, the growing colonies are identified in a systematic way.

VI. Antibiotic sensitivity test is done for the isolated bacteria.

VII. When T.B. is suspected, 5 successive morning samples are subjected to Z-N staining and culture on L.J. medium.

Sterile pyuria: i.e. no growth on ordinary culture media despite the presence of pus cells in urine (more than 10/HPF). This may be due to:

1. Renal tuberculosis.
2. Female genital tract infections.
3. Non-gonococcal urethritis in male patients such as *Chlamydia trachomatis*.
4. Infection by anaerobic bacteria, genital Mycoplasma or L-forms of bacteria.
5. Recent antimicrobial chemotherapy.
6. Prostatitis.
7. Non-infectious conditions: Neoplasia of the renal tract, renal calculi, catheterization and fever in children independent of the cause.

Prostatitis

Prostatitis must always be considered in the differential diagnosis of UTI. The male urethra courses through the centre of the prostate on its way to the bladder. This relationship allows for invasion of urinary tract pathogens into the prostate, causing acute bacterial prostatitis. Patients with prostatitis will present with symptoms of UTI and a large tender prostate. The enlarged prostate may also cause symptoms of bladder obstruction by physically blocking urine flow. The agents that cause prostatitis are the same Gram-negative agents that cause UTI as well as *Chlamydia*, *Mycoplasma* and *Ureaplasma* species.

For the diagnosis of prostatitis, expressed prostatic secretion or urine may be collected after massage of the prostate via the rectum because this is said to release any sequestered bacteria or inflammatory cells from this site.

Urethritis

Urethritis is an inflammatory condition that can be infectious or post-traumatic. Urethritis is predominantly a disease of adult men. Infectious causes of urethritis are typically sexually transmitted.

Categories of urethritis include:

1. **Gonococcal urethritis** due to *Neisseria gonorrhoeae* (the most common cause).
2. **Nongonococcal urethritis** due to:
 - *Chlamydia trachomatis* (serotypes D-K).
 - *Ureaplasma urealyticum*.
 - *Mycoplasma hominis*.
 - *Trichomonas vaginalis*.
3. Pyogenic bacteria as *E. coli*, staphylococci and streptococci. They usually occur in the presence of urethral stricture or post-traumatic following catheterization, instrumentation or foreign body insertion.
4. Intrameatal warts by human papilloma virus.
5. Intrameatal ulcer by Herpes simplex virus.
6. Intrameatal chancre.
7. Adenovirus.

Skin and Soft Tissue Infections

Lesions in skin are designated as macules, papules, vesicles, bullae, or pustules

A) Localized infections of the skin

Infections	Organism
Bacterial	
Folliculitis, furuncles, carbuncles, or abscess	<i>S. aureus</i>
Cellulitis, erysipelas, impetigo (pyoderma), necrotizing fasciitis	<i>S. pyogenes</i>
Malignant pustule	<i>Bacillus anthracis</i>
Actinomycotic mycetoma	Actinomycetes
Fungal	
Superficial mycoses: <i>Pityriasis versicolor</i>	Yeasts: <i>Malassezia furfur</i>
Cutaneous candidiasis	Yeasts: <i>Candida</i> species
Cutaneous mycoses	Dermatophytes
Eumycotic mycetoma	<i>Madurella</i> and <i>Allescheria</i> species
Viral	
Skin warts	Human papillomaviruses (HPVs)
Herpes simplex	Herpes simplex viruses 1 or 2
Zoster (shingles)	Varicella-zoster virus (VZV)
Roseola infantum	Human herpes viruses 6 (HHV6)
Erythema infectiosum	Human parvovirus B 19
Molluscum contagiosum	Molluscum contagiosum virus (MCV)
Smallpox	Variola virus

B) Systemic diseases with skin rash

Disease	Organism
Bacterial	
Meningococcaemia	<i>Neisseria meningitides</i>
Toxic shock syndrome	<i>S. aureus</i> , <i>S. pyogenes</i>
Scarlet fever	<i>S. pyogenes</i>
Rocky Mountain Spotted Fever	<i>Rickettsia rickettsii</i>
Secondary syphilis	<i>Treponema pallidum</i>
Lyme disease	<i>Borrelia burgdorferi</i>
Staphylococcal scalded skin syndrome	<i>S. aureus</i>
Viral	
Chickenpox	Varicella-zoster virus (VZV)
Measles	Rubeola virus
German measles	Rubella virus
Febrile illness	Echoviruses
AIDS	Human immunodeficiency virus
Smallpox	Variola virus

Pyrexia of Undetermined Origin (PUO)

A patient who presents with pyrexia as a predominant clinical feature, for at least 10 days, without an obvious cause is considered as a case of PUO. Infective causes are responsible for 75% of cases of "acute PUO".

Major causes of PUO

1. Infections.
2. Neoplasms.
3. Autoimmune diseases as systemic lupus and rheumatic fever.
4. Drug induced fever.
5. Granulomatous disease.

Infective causes of PUO

A- Non-specific causes:

1. Cryptic abscesses in: liver, abdomen, pelvis and retroperitoneal or mediastinal sites.
2. Infective endocarditis.
3. Urinary tract infections.
4. Ear, sinus or dental infections.

B- Specific causes:

1.	Bacterial disease	• Tuberculosis • Typhoid • Brucellosis • Typhus	• Leptospirosis • Relapsing fever • Q fever • Nocardiosis
2.	Viral diseases	• Hepatitis A or B • Cytomegalovirus disease • Lassa fever	• Yellow fever • HIV infection (AIDS) • Infectious mononucleosis
3.	Protozoal diseases	• Malaria • Amoebiasis	• Toxoplasmosis • Trypanosomiasis
4.	Fungal diseases	• Cryptococcosis • Candidiasis • Pneumocystis	• Histoplasmosis • Aspergillosis

Diagnosis of PUO

- I. Detailed history.
- II. Complete physical examination.
- III. Temperature chart to determine the pattern of fever.
- IV. Laboratory tests as:
 1. Microbial test
 - Blood culture
 - Blood smears for parasites, e.g., malaria.
 - Urine and faeces analysis.
 - Serology, preferably paired sera to look for rising antibody titre. Examples of diseases that can be diagnosed serologically include: typhoid fever (Widal), rheumatic fever (ASO), brucellosis, Q fever, typhus, leptospira, infectious mononucleosis (Paul-Bunnell or monospot), cytomegalovirus, HBV (HBsAg), HIV, ... etc.
 2. Haematological test: Complete blood picture and erythrocyte sedimentation rate.

Infective Endocarditis

The common bacteria involved are:

1. Viridans streptococci (subacute bacterial endocarditis).
2. Coagulase-negative staphylococci (particularly with prosthetic valve).
3. *Streptococcus pyogenes*.
4. *Staphylococcus aureus*.
5. *Enterococcus* species.
6. *Haemophilus* species.
7. *Coxiella burnetii* (Q fever agent).

Blood Culture

In most bacterial infections of the blood (bacteraemia), the organisms are not numerous and, so, direct plating of a drop of the patient's blood will usually give a negative result. Therefore, for diagnosis of such infections, it is recommended that 5-10 ml of venous blood be obtained for each culture in an adult and is added to 50-100 ml broth. The culture should be incubated anaerobic and aerobic at 37°C. Subcultures are done on suitable solid media every 48 hours and growing organisms are identified in a systematic way.

For better results, repeated blood samples should be collected from different sites preferably before the start of antibiotic therapy. Therefore, cultures of three sets of blood withdrawn within a 24- to 48-h period are sufficient to establish a diagnosis of bacteraemia. Culture-negative is best defined when negative blood cultures (at least three venous blood cultures) are obtained repetitively.

The large volume of broth used in blood culture has the following advantages:

1. It reduces the concentration of natural antimicrobial constituents in the blood to a sub-inhibitory level.
2. Allows the growth of the few organisms present in the blood sample in a small volume so that the drop used in the subsequent subculture will contain a number of organisms sufficient to be demonstrated and identified.
3. In patients under antibiotic therapy, neutralization or diminution of the presence of antibiotics in blood may be accomplished by different methods.

The most common infectious diseases that can be diagnosed by blood culture include:

- Enteric Fever.
- Brucellosis.
- Endocarditis.
- Meningitis.
- Puerperal sepsis.
- Necrotizing fasciitis caused by *Streptococcus pyogenes*.
- Toxic shock syndrome caused by *Streptococcus pyogenes*.

Chapter 22

TRANSMISSION BASED INFECTIONS

Airborne (aerosol) infections

Airborne transmission occurs by dissemination of either airborne **droplet nuclei** (small-particles [$\leq 5 \mu\text{m}$ in size] produced from evaporated droplets) or **dust particles** containing the infectious agent. Microorganisms carried in this manner may remain suspended in the air for long periods of time and can be dispersed widely by air currents. The particles may become inhaled by or deposited on a susceptible host within the same room or over a **longer distance** from the source. The most important infections transmitted by this way include:

- ① Pulmonary tuberculosis caused by *Mycobacterium tuberculosis*.
- ② Measles.
- ③ Varicella Zoster virus (VZV) infections.
- ④ Q fever caused by *Coxiella burnetii*.
- ⑤ Others: *Brucella* spp., *Legionella pneumophila* and *Bacillus anthracis*.

Droplet infections

Droplet transmission involves contact of the conjunctivae or the mucous membranes of the nose or mouth of a susceptible person with **large droplets** ($> 5 \mu\text{m}$ in size) containing microorganisms generated from a person who has a clinical disease or who is a carrier of the microorganism. Droplets are generated during coughing, sneezing, or talking. Transmission via large droplets requires **close contact** between the source and the recipients, because droplets do not remain suspended in the air and generally travel only short distances, usually 3 feet or less, through the air. The most important infections transmitted by this way include:

- ① *Haemophilus influenzae* type b.
- ② *Neisseria meningitidis*.
- ③ *Streptococcus pyogenes*.
- ④ *Mycoplasma pneumoniae*.
5. Other serious bacterial respiratory infections including: Diphtheria (pharyngeal), pertussis and pneumonic plague.
6. Serious viral infections including:
 - Influenza.
 - Rubella (German measles).
 - Others: Adenovirus, mumps, parvovirus B19 and SARS virus.

Sexually Transmitted Diseases (STDs)

The most common causes are:

1. Bacterial

- *Neisseria gonorrhoeae*
- *Treponema pallidum*
- *Haemophilus ducrey*
- *Chlamydia trachomatis* (serotypes L1,2,3)
- *Chlamydia trachomatis* (serotypes D-K)
- *Ureaplasma urealyticum*

2. Viral

- Herpes simplex viruses *HSV II*
- Human papillomaviruses
- Human immunodeficiency virus *HIV*
- Hepatitis B virus
- Molluscum contagiosum virus (MCV)

N.B.:

1. The patient may have more than one STDs at the same time.
2. The causative organisms are delicate and die quickly outside the body.

Diseases Transmitted from Mother to Foetus

I. Pre-natal (congenital) infections: are infections that can be transmitted to the foetus by transplacental route. They include:

Viruses

- Rubella.
- ⊗ Cytomegalovirus
- ⊗ Varicella zoster virus.
- ⊗ Human immunodeficiency virus.
- Parvovirus B19.

Bacteria

- *Treponema pallidum*.
- *Listeria monocytogenes*.

Protozoa

- ⊗ *Toxoplasma gondii*.

II. Peri-natal infections: are infections transmitted during birth. They include:

Bacteria

- *Neisseria gonorrhoeae* (ophthalmia neonatorum).
- *Chlamydia trachomatis* (neonatal conjunctivitis, pneumonia).
- *Streptococcus agalactiae* (neonatal sepsis and meningitis).
- *Listeria monocytogenes* (listeriosis; septicaemia, meningitis).

Viruses

- Hepatitis B virus.
- Human immunodeficiency virus.
- Herpes simplex.
- Cytomegalovirus.

Diagnosis

Serologic tests are considered the most important to confirm clinically suspected infection. Serologic evaluation should be done for both mother and child focusing on potential congenital infection particularly what is known as ToRCH profile. ToRCH profile refers to **T**oxoplasmosis, **R**ubella, **C**ytomegalovirus and **H**erpes. Demonstration of specific IgM antibodies or a 4-fold rise of specific IgG antibodies indicates recent infection.

Microorganisms Transmitted by Blood Transfusion

1. Hepatitis viruses B, C, D and G.
2. HIV types 1 and 2.
3. Cytomegalovirus (CMV).
4. Epstein-Barr virus (EBV).
5. Human T-cell leukaemia virus (HTLV-1).
6. Human parvovirus B19 (HPV-B19).
7. Brucella.
8. *Treponema pallidum*.
9. Malaria parasite.

- CMV, EBV, HTLV and malaria are present in the cellular components of blood, thus, are not transmitted by plasma.
- *Trep. pallidum* and malaria die when stored at 4°C within 3-5 days, so are not transmitted by stored blood.
- Blood free from infectious agent may be contaminated by bacteria during withdrawal from the donor or from the environment.

Zoonotic Diseases

These are diseases mainly of vertebrate animals that can also be transmitted to man. In many instances, the animals may not have obvious illness but may still be infective for man. Man may be end host (no man-man transmission).

Some **occupations** have a higher incidence of reservoir contact such as veterinarians, slaughterhouse and carpet mill workers, farmers and butchers.

Transmission routes include:

1. Direct contact with infected tissues or infectious fomites.
2. Ingestion of infectious meat or milk.
3. Inhalation of infectious aerosols.
4. Inoculation by the bite of an infected reservoir or vector animal.



Zoonotic infections: The most common causes are:

1) **Bacterial**

- Brucellosis (*Brucella* species).
- Tuberculosis (*Mycobacterium bovis*).
- Yersiniosis (*Yersinia enterocolitica*).
- Listeriosis (*Listeria monocytogenes*).
- Anthrax (*Bacillus anthracis*).
- Leptospirosis (*Leptospira interrogans*).
- Q fever (*Coxiella burnetii*).
- Bubonic plague (*Yersinia pestis*).
- Lyme disease (*Borrelia burgdorferi*).
- Endemic relapsing fever (*Borrelia duttoni*).
- Rocky Mountain Spotted Fever (*Rickettsia rickettsii*).
- Endemic typhus (*Rickettsia typhi*).

2) **Viral:**

- Rabies.
- Yellow fever.
- Rift valley fever virus.
- Haemorrhagic fever viruses (Hantavirus and Ebola fever viruses).

Pathogens Transmitted by Milk and Milk Products

1. Pathogens from diseased animals and **excreted** in milk and can be eradicated by pasteurization:

- M. bovis. → T.B.
- Brucella abortus and melitensis.
- Coxiella burnetii. → Q fever

2. Pathogens that may contaminate milk:

a- Bacteria:

- *Salmonella* Typhi and *S. Paratyphi*.
- *Shigella* species.
- *Campylobacter* species.
- *Staph. aureus*.
- *Strept. pyogenes*.
- Verotoxin producing *E. coli*.
- *Vibrio cholerae*.

b- Viruses:

- Poliomyelitis and enteroviruses.
- Hepatitis A and E.
- Rotavirus.

c- Aflatoxin produced by *Aspergillus flavus*.



Biological Warfare and Bioterrorism

Bioterrorism is defined as the use of biological agents to inflict disease and/or death on humans, animals or plants. The biological agent used include microorganisms as well as microbial toxins, plants, and animals. They are categorized as follows:

Category (A) characterized by being easily disseminated and cause high mortality.

- Bacteria* {
1. Anthrax.
 2. Smallpox. ————— *Virus*
 3. Botulism.
 4. Hemorrhagic fever viruses.
 5. Plague.

Category B

These agents are moderately easily disseminated and cause moderate morbidity and low mortality.

1. Brucellosis.
2. Q Fever.
3. Staphylococcus toxin.

Category C

These agents include emerging pathogens that could be engineered for mass dissemination in the future because of availability, e.g. drug-resistant TB, hantavirus (mice), and yellow fever (mosquito).

Chapter 23

VACCINATION

Vaccination involves the administration of microorganisms or their products (in an unharmed state) into a host. The aim is to induce an appropriate immune response which is capable of protecting the host when he is exposed later to a virulent form of the same pathogen.

The form of the vaccine determines the kind of immune response produced. **Non-living vaccines** cannot enter the MHC class I pathway and, therefore, cannot induce cytotoxic T-cells. **Live vaccines**, on the other hand, have access to both MHC class I and II pathways and can, therefore, induce both cytotoxic and helper T-cells. Thus, live vaccines are more effective in inducing a better overall immune response. However, they can not be used in pregnant women and immunocompromised hosts. They also require refrigeration which makes them more expensive and difficult to use in underdeveloped countries.

Vaccines, in general, can be classified as follows:

A. Bacterial Vaccines

1. Intact Bacteria:

- a- **Killed**: using organisms killed by heat or chemicals, e.g. pertussis vaccine, typhoid vaccine (TAB) and cholera vaccine.
- b- **Live attenuated**: prepared by frequent subculture on artificial suboptimal media, e.g. Bacille Calmette-Guerin (BCG) vaccine against tuberculosis.

2. Bacterial Materials:

- a- **Structural Components**: Such as the capsule of bacteria, which induce the production of anti-capsular antibodies that neutralize the antiphagocytic effect of the capsule, e.g. pneumococcal polysaccharide vaccine, Haemophilus influenzae type b vaccine (Hib vaccine) and Neisseria meningitidis vaccine.
- b- **Toxoids**: Non-toxic, immunogenic preparations produced from exotoxins by the use of formalin. They induce antitoxic antibodies. Well known toxoids are diphtheria and tetanus toxoids.

B- Viral Vaccines

1. Intact virus

- a- **Killed (inactivated)**: Inactivation is done by formalin or ultraviolet light. Examples are Salk polio vaccine and rabies vaccine.
- b- **Attenuated**: Attenuation is done by repeated subculture in tissue culture or serial passage in various animal hosts. Examples are Sabin polio vaccine, mumps, measles and rubella vaccine (MMR).

2. Viral Components

- a- **Isolated viral antigens**: Hepatitis B vaccine is composed of hepatitis B surface antigen (HbsAg).



b- Disrupted or split vaccine preparations such as influenza vaccine containing the haemagglutinin and neuraminidase.

Progress in molecular biology as well as a better understanding of immune response to pathogens has led to **new techniques of vaccine production**:

1. **New Methods of attenuation of virus** by deletion of genes responsible for virulence which prevents their reversion to virulence.
2. **Recombinant vector vaccines (live vector vaccines)**: The genes encoding antigens of interest are isolated and inserted into a known attenuated viral or bacterial vaccine strain (Vaccinia virus or BCG). This is then given as a live vaccine.
3. **Synthetic peptide vaccines**: This involves sequencing of peptide genes, followed by synthesis of peptides and their use for vaccination.
4. **Anti-idiotypic antibody vaccines**: Anti-idiotypic antibodies are directed at the hypervariable region of the antibody. They mimic epitopes of the original antigen and can be used in immunization instead of the antigen.
5. **DNA vaccines**: This method involves the injection of naked DNA plasmids encoding immunogenic proteins of interest. It is still experimental but is very promising. The protein is produced in the body for a long time and is a constant source of stimulation to both arms of the immune system without a need for booster doses.
6. **Edible vaccines**: Scientists have been able to insert genes from pathogens into edible plants, such as bananas, where they replicate. The plant can be eaten directly as a source of vaccine at a low cost. Animal studies have been done and in the future these edible vaccine may be used for humans.

The schedule of immunization of children in Egypt is as follows:

- Birth-1 month: BCG vaccine.
- 2 months: diphtheria-pertussis-tetanus (DPT), oral polio vaccine (OPV) and Hepatitis B vaccine.
- 4 months: DPT, OPV, and Hepatitis B vaccine.
- 6 months: DPT, OPV, and Hepatitis B vaccine.
- 18 months: OPV, DPT.
- 12-15 months: MMR vaccine first dose.
- 4-6 Years: DT and polio vaccines.

Other routine childhood vaccines recommended by the WHO include Hib vaccine at 2, 4 and 6 months, and capsular polysaccharide antigen of group A, C, Y and W-135 of *Neisseria meningitidis*. The latter is given to children above 2 years of age and can be given to exposed adults as army recruits.

JK
Vaccine
17/11/17